WO 01/45728 PCT/US00/35516

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 June 2001 (28.06.2001)

PCT

(10) International Publication Number
WO 01/45728 A2

(51) International Patent Classification[7]: A61K 38/08, 39/00, 39/385, 39/39, C07H 21/04, C07K 14/435

(21) International Application Number: PCT/US00/35516

(22) International Filing Date:
20 December 2000 (20.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/171,312 21 December 1999 (21.12.1999) US
09/633,364 7 August 2000 (07.08.2000) US

(71) Applicant: **EPIMMUNE INC.** [US/US]; 5820 Nancy Ridge Drive, San Diego, CA 92121 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FIKES, John** [US/US]; 4671 Robbins Street, San Diego, CA 92122 (US). **SETTE, Alessandro** [IT/US]; 5551 Linda Rosa Avenue, La Jolla, Ca California 92037 (US). **SIDNEY, John** [US/US]; 4218 Corte delaa Siena, San Diego, CA 92130 (US). **SOUTHWOOD, Scott** [US/US]; 19679 Strathmore Drive, Santee, CA 92071 (US). **CHESNUT, Robert** [US/US]; 1473 Kings Cross Drive, Cardiff-by-the-Sea, CA 92007 (US). **CELIS, Esteban** [US/US]; 3683 Wright Road S.W., Rochester, MN 55902 (US). **KEOGH, Elissa** [US/US]; 799 Caminito Dia #2, San Diego, CA 92122 (US).

(74) Agents: **LOCKYER, Jean, M.** et al.; Townsend Townsend and Crew LLP, Two Embarcadero Center, Eighth Floor, San Francisco, CA 94111 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With declaration under Article 17(2)(a); without abstract; title not checked by the International Searching Authority.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/45728 A2

(54) Title: INDUCING CELLULAR IMMUNE RESPONSES TO PROSTATE CANCER ANTIGENS USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

(57) Abstract:

INDUCING CELLULAR IMMUNE RESPONSES TO PROSTATE CANCER ANTIGENS USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

I. BACKGROUND OF THE INVENTION

A growing body of evidence suggests that cytotoxic T lymphocytes (CTL) are important in the immune response to tumor cells. CTL recognize peptide epitopes in the context of HLA class I molecules that are expressed on the surface of almost all nucleated cells. Following intracellular processing of endogenously synthesized tumor antigens, antigen-derived peptide epitopes bind to class I HLA molecules in the endoplasmic reticulum, and the resulting complex is then transported to the cell surface. CTL recognize the peptide-HLA class I complex, which then results in the destruction of the cell bearing the HLA-peptide complex directly by the CTL and/or via the activation of non-destructive mechanisms, *e.g.*, activation of ~~lymphokines such as tumor necrosis~~ factor-α (TNF- α) or interferon-γ (IFNγ) ~~which~~ enhance the immune response and facilitate the destruction of the tumor cell.

Tumor-specific helper T lymphocytes (HTLs) are also known to be important for maintaining effective antitumor immunity. Their role in antitumor immunity has been demonstrated in animal models in which these cells not only serve to provide help for induction of CTL and antibody responses, but also provide effector functions, which are mediated by direct cell contact and also by secretion of lymphokines (*e.g.*, IFNγ and TNF- α).

A fundamental challenge in the development of an efficacious tumor vaccine is immune suppression or tolerance that can occur. There is therefore a need to establish vaccine embodiments that elicit immune responses of sufficient breadth and vigor to prevent progression and/or clear the tumor.

The epitope approach, as we have described, represents a solution to this challenge, in that it allows the incorporation of various CTL, HTL, and antibody (if desired) epitopes from discrete regions of one or more target tumor-associated antigens (TAAs) in a single vaccine composition. Such a composition may simultaneously target multiple dominant and subdominant epitopes and thereby be used to achieve effective immunization in a diverse population.

Prostate cancer is the most common malignancy in men. Current therapies, *i.e.*, chemotherapy combined with androgen blockade, antiandrogen withdrawal, and other secondary hormonal therapies, have met with limited success. Thus, there is a need to develop more efficacious therapies. The multiepitopic immunotherapy vaccine compositions of the present invention fulfill this need.

Antigens that are associated with prostate cancer include, but are not limited to, prostate specific antigen (PSA), prostate specific membrane antigen (PSM), prostatic acid phosphatase (PAP), and human kallikrein2 (hK2 or HuK2). These antigens represent important antigen targets for the polyepitopic vaccine compositions of the invention.

PSM is also an important candidate for prostate cancer therapy. It is a Type II membrane protein that is expressed at high levels on prostate adenocarcinomas. The levels of expression increase on

metastases and in carcinomas that are refractory to hormone therapy. PSM is not generally present on normal tissues, although low levels have been detected in the colonic crypts and in the duodenum, and PSM can be detected in normal male serum and seminal fluid (*see, e.g.*, Silver *et al.*, *Clin. Cancer Res.* 3:81-85, 1997). CTL responses to PSM have also been documented (*see, e.g.*, Murphy *et al.*, *Prostate* 29:371-380, 1996; and Salgaller *et al.*, *Prostate* 35:144-151, 1998).

PAP is a tissue-specific differentiation antigen that is secreted exclusively by cells in the prostate (*see, e.g.*, Lam *et al.*, *Prostate* 15:13-21, 1989). It can be detected in serum and levels are increased in patients with prostate carcinoma (*see, e.g.*, Jacobs *et al.*, *Curr. Probl. Cancer* 15:299-360, 1991). The PAP protein sequence has, at best, a 49% sequence homology with other acid phosphatases with the homologous regions distributed throughout the protein. Accordingly, PAP-specific epitopes can be identified and several different CTL epitopes have been described (*see, e.g.*, Peshwa *et al.*, *Prostate* 36:129-138, 1998).

The hK2 protein is functionally a serine protease involved in posttranslational processing of polypeptides. It is expressed by prostate epithelia exclusively, and is found in both benign and malignant prostate cancer tissue. Although it is expressed in 50% of normal prostate cells, the percentage of cells expressing hK2 is increased in adenocarcinomas and prostatic intraepithelial neoplasia (PIN) (*see, e.g.*, Darson *et al.*, *Urology* 49:857-862, 1997). Based on the preferential expression of this antigen on prostate cancer cells, hK2 is also an important target for immunotherapy.

Prostate-specific antigen (PSA), also referred to as hK3, is a secreted serine protease and a member of the kallikrein family of proteins. The PSA gene is 80% homologous with the hK2 gene, however, tissue expression of hK2 is regulated independently of PSA (*see, e.g.*, Darson *et al.*, *Urology* 49:857-862, 1997). Expression of PSA is restricted to prostate epithelial cells, both benign and malignant. The antigen can be detected in the serum of most prostate cancer patients and in seminal plasma. Several T cell epitopes from PSA have been identified and have been found to be immmnogenic, and antibody responses have been reported in patients (*see, e.g.*, Correale *et al.*, *J. Immunol.* 161:3186, 1998; and Alexander *et al.*, *Urology* 51:150-157, 1998). Thus, based on its prostate-restricted expression and ability to stimulate immune responses, PSA is an attractive target for immunotherapy of prostate cancer.

The information provided in this section is intended to disclose the presently understood state of the art as of the filing date of the present application. Information is included in this section which was generated subsequent to the priority date of this application. Accordingly, information in this section is not intended, in any way, to delineate the priority date for the invention.

II. SUMMARY OF THE INVENTION

This invention applies our knowledge of the mechanisms by which antigen is recognized by T cells, for example, to develop epitope-based vaccines directed towards TAAs. More specifically, this application identifies epitopes for inclusion in diagnostic and/or pharmaceutical compositions and methods of use of the epitopes for the evaluation of immune responses and for the treatment and/or prevention of cancer.

The use of epitope-based vaccines has several advantages over current vaccines, particularly when compared to the use of whole antigens in vaccine compositions. For example,

immunosuppressive epitopes that may be present in whole antigens can be avoided with the use of epitope-based vaccines. Such immunosuppressive epitopes may, *e.g.*, correspond to immunodominant epitopes in whole antigens, which may be avoided by selecting peptide epitopes from non-dominant regions (*see, e.g.*, Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996).

An additional advantage of an epitope-based vaccine approach is the ability to combine selected epitopes (CTL and HTL), and further, to modify the composition of the epitopes, achieving, for example, enhanced immunogenicity. Accordingly, the immune response can be modulated, as appropriate, for the target disease. Similar engineering of the response is not possible with traditional approaches.

Another major benefit of epitope-based immune-stimulating vaccines is their safety. The possible pathological side effects caused by infectious agents or whole protein antigens, which might have their own intrinsic biological activity, is eliminated.

An epitope-based vaccine also provides the ability to direct and focus an immune response to multiple selected antigens from the same pathogen (a "pathogen" may be an infectious agent or a tumor-associated molecule). Thus, patient-by-patient variability in the immune response to a particular pathogen may be alleviated by inclusion of epitopes from multiple antigens from the pathogen in a vaccine composition.

Furthermore, an epitope-based anti-tumor vaccine also provides the opportunity to combine epitopes derived from multiple tumor-associated molecules. This capability can therefore address the problem of tumor-to tumor variability that arises when developing a broadly targeted anti-tumor vaccine for a given tumor type and can also reduce the likelihood of tumor escape due to antigen loss. For example, prostate cancer cells in one patient may express target TAAs that differ from the prostate cancer cells in another patient. Epitopes derived from multiple TAAs can be included in a polyepitopic vaccine that will target both prostate cancers.

One of the most formidable obstacles to the development of broadly efficacious epitope-based immunotherapeutics, however, has been the extreme polymorphism of HLA molecules. To date, effective non-genetically biased coverage of a population has been a task of considerable complexity; such coverage has required that epitopes be used that are specific for HLA molecules corresponding to each individual HLA allele. Impractically large numbers of epitopes would therefore have to be used in order to cover ethnically diverse populations. Thus, there has existed a need for peptide epitopes that are bound by multiple HLA antigen molecules for use in epitope-based vaccines. The greater the number of HLA antigen molecules bound, the greater the breadth of population coverage by the vaccine.

Furthermore, as described herein in greater detail, a need has existed to modulate peptide binding properties, *e.g.*, so that peptides that are able to bind to multiple HLA molecules do so with an affinity that will stimulate an immune response. Identification of epitopes restricted by more than one HLA allele at an affinity that correlates with immunogenicity is important to provide thorough population coverage, and to allow the elicitation of responses of sufficient vigor to prevent or clear an infection in a diverse segment of the population. Such a response can also target a broad array of epitopes. The technology disclosed herein provides for such favored immune responses.

In a preferred embodiment, epitopes for inclusion in vaccine compositions of the invention are selected by a process whereby protein sequences of known antigens are evaluated for the

presence of motif or supermotif-bearing epitopes. Peptides corresponding to a motif- or supermotif-bearing epitope are then synthesized and tested for the ability to bind to the HLA molecule that recognizes the selected motif. Those peptides that bind at an intermediate or high affinity *i.e.*, an IC_{50} (or a K_D value) of about 500 nM or less for HLA class I molecules or an IC_{50} of about 1000 nM or less for HLA class II molecules, are further evaluated for their ability to induce a CTL or HTL response. Immunogenic peptide epitopes are selected for inclusion in vaccine compositions.

Supermotif-bearing peptides may additionally be tested for the ability to bind to multiple alleles within the HLA supertype family. Moreover, peptide epitopes may be analoged to modify binding affinity and/or the ability to bind to multiple alleles within an HLA supertype.

The invention also includes embodiments comprising methods for monitoring or evaluating an immune response to a TAA in a patient having a known HLA-type. Such methods comprise incubating a T lymphocyte sample from the patient with a peptide composition comprising a TAA epitope that has an amino acid sequence comprising a supermotif or motif and which binds the product of at least one HLA allele present in the patient, and detecting for the presence of a T lymphocyte that binds to the peptide. A CTL peptide epitope may, for example, be used as a component of a tetrameric complex for this type of analysis.

An alternative modality for defining the peptide epitopes in accordance with the invention is to recite the physical properties, such as length; primary structure; or charge, which are correlated with binding to a particular allele-specific HLA molecule or group of allele-specific HLA molecules. A further modality for defining peptide epitopes is to recite the physical properties of an HLA binding pocket, or properties shared by several allele-specific HLA binding pockets (*e.g.* pocket configuration and charge distribution) and reciting that the peptide epitope fits and binds to the pocket or pockets.

As will be apparent from the discussion below, other methods and embodiments are also contemplated. Further, novel synthetic peptides produced by any of the methods described herein are also part of the invention.

III. BRIEF DESCRIPTION OF THE FIGURES

not applicable

IV. DETAILED DESCRIPTION OF THE INVENTION

The peptide epitopes and corresponding nucleic acid compositions of the present invention are useful for stimulating an immune response to a TAA by stimulating the production of CTL or HTL responses. The peptide epitopes, which are derived directly or indirectly from native TAA protein amino acid sequences, are able to bind to HLA molecules and stimulate an immune response to the TAA. The complete sequence of the TAA proteins to be analyzed can be obtained from GenBank. Peptide epitopes and analogs thereof can also be readily determined from sequence information that may subsequently be discovered for heretofore unknown variants of particular TAAs, as will be clear from the disclosure provided below.

A list of target TAAs includes, but is not limited to, the following antigens: MAGE 1, MAGE 2, MAGE 3, MAGE-11, MAGE-A10, BAGE, GAGE, RAGE, MAGE-C1, LAGE-1, CAG-3,

DAM, MUC1, MUC2, MUC18, NY-ESO-1, MUM-1, CDK4, BRCA2, NY-LU-1, NY-LU-7, NY-LU-12, CASP8, RAS, KIAA-2-5, SCCs, p53, p73, CEA, Her 2/neu, Melan-A, gp100, tyrosinase, TRP2, gp75/TRP1, kallikrein, PSM, PAP, PSA, PT1-1, B-catenin, PRAME, Telomerase, FAK, cyclin D1 protein, NOEY2, EGF-R, SART-1, CAPB, HPVE7, p15, Folate receptor CDC27, PAGE-1, and PAGE-4. Epitopes derived from these antigens may be used in combination with one another to target a specific tumor type, *e.g.*, prostate tumors, or to target multiple types of tumors.

The peptide epitopes of the invention have been identified in a number of ways, as will be discussed below. Also discussed in greater detail is that analog peptides have been derived and the binding activity for HLA molecules modulated by modifying specific amino acid residues to create peptide analogs exhibiting altered immmunogenicity. Further, the present invention provides compositions and combinations of compositions that enable epitope-based vaccines that are capable of interacting with HLA molecules encoded by various genetic alleles to provide broader population coverage than prior vaccines.

IV.A. Definitions

The invention can be better understood with reference to the following definitions, which are listed alphabetically:

A "construct" as used herein generally denotes a composition that does not occur in nature. A construct can be produced by synthetic technologies, *e.g.*, recombinant DNA preparation and expression or chemical synthetic techniques for nucleic or amino acids. A construct can also be produced by the addition or affiliation of one material with another such that the result is not found in nature in that form.

A "computer" or "computer system" generally includes: a processor; at least one information storage/retrieval apparatus such as, for example, a hard drive, a disk drive or a tape drive; at least one input apparatus such as, for example, a keyboard, a mouse, a touch screen, or a microphone; and display structure. Additionally, the computer may include a communication channel in communication with a network. Such a computer may include more or less than what is listed above.

"Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

A "cryptic epitope" elicits a response by immunization with an isolated peptide, but the response is not cross-reactive *in vitro* when intact whole protein which comprises the epitope is used as an antigen.

A "dominant epitope" is an epitope that induces an immune response upon immunization with a whole native antigen (see, *e.g.*, Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993). Such a response is cross-reactive *in vitro* with an isolated peptide epitope.

With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site

recognized by an immunoglobulin, T cell receptor or HLA molecule. Throughout this disclosure epitope and peptide are often used interchangeably.

It is to be appreciated that protein or peptide molecules that comprise an epitope of the invention as well as additional amino acid(s) are within the bounds of the invention. In certain embodiments, there is a limitation on the length of a peptide of the invention which is not otherwise a construct as defined herein. An embodiment that is length-limited occurs when the protein/peptide comprising an epitope of the invention comprises a region (i.e., a contiguous series of amino acids) having 100% identity with a native sequence. In order to avoid a recited definition of epitope from reading, *e.g.*, on whole natural molecules, the length of any region that has 100% identity with a native peptide sequence is limited. Thus, for a peptide comprising an epitope of the invention and a region with 100% identity with a native peptide sequence (and which is not otherwise a construct), the region with 100% identity to a native sequence generally has a length of: less than or equal to 600 amino acids, often less than or equal to 500 amino acids, often less than or equal to 400 amino acids, often less than or equal to 250 amino acids, often less than or equal to 100 amino acids, often less than or equal to 85 amino acids, often less than or equal to 75 amino acids, often less than or equal to 65 amino acids, and often less than or equal to 50 amino acids. In certain embodiments, an "epitope" of the invention which is not a construct is comprised by a peptide having a region with less than 51 amino acids that has 100% identity to a native peptide sequence, in any increment of (50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5) down to 5 amino acids.

Certain peptide or protein sequences longer than 600 amino acids are within the scope of the invention. Such longer sequences are within the scope of the invention so long as they do not comprise any contiguous sequence of more than 600 amino acids that have 100% identity with a native peptide sequence, or if longer than 600 amino acids, they are a construct. For any peptide that has five contiguous residues or less that correspond to a native sequence, there is no limitation on the maximal length of that peptide in order to fall within the scope of the invention. It is presently preferred that a CTL epitope of the invention be less than 600 residues long in any increment down to eight amino acid residues.

"Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (*see, e.g.*, Stites, *et al.*, IMMUNOLOGY, 8TH ED., Lange Publishing, Los Altos, CA, 1994).

An "HLA supertype or family", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like molecules (where xx denotes a particular HLA type), are synonyms.

Throughout this disclosure, results are expressed in terms of "IC_{50}'s." IC_{50} is the concentration of peptide in a binding assay at which 50% inhibition of binding of a reference peptide is observed. Given the conditions in which the assays are run (*i.e.*, limiting HLA proteins and labeled peptide concentrations), these values approximate K_D values. Assays for determining binding are described in detail, *e.g.*, in PCT publications WO 94/20127 and WO 94/03205. It should be noted that IC_{50} values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used

(*e.g.*, HLA preparation, *etc.*). For example, excessive concentrations of HLA molecules will increase the apparent measured IC_{50} of a given ligand.

Alternatively, binding is expressed relative to a reference peptide. Although as a particular assay becomes more, or less, sensitive, the IC_{50}'s of the peptides tested may change somewhat, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC_{50} of the reference peptide increases 10-fold, the IC_{50} values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC_{50}, relative to the IC_{50} of a standard peptide.

Binding may also be determined using other assay systems including those using: live cells (*e.g.*, Ceppellini *et al.*, *Nature* 339:392, 1989; Christnick *et al.*, *Nature* 352:67, 1991; Busch *et al.*, *Int. Immunol.* 2:443, 19990; Hill *et al.*, *J. Immunol.* 147:189, 1991; del Guercio *et al.*, *J. Immunol.* 154:685, 1995), cell free systems using detergent lysates (*e.g.*, Cerundolo *et al.*, *J. Immunol.* 21:2069, 1991), immobilized purified MHC (*e.g.*, Hill *et al.*, *J. Immunol.* 152, 2890, 1994; Marshall *et al.*, *J. Immunol.* 152:4946, 1994), ELISA systems (*e.g.*, Reay *et al.*, *EMBO J.* 11:2829, 1992), surface plasmon resonance (*e.g.*, Khilko *et al.*, *J. Biol. Chem.* 268:15425, 1993); high flux soluble phase assays (Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994), and measurement of class I MHC stabilization or assembly (*e.g.*, Ljunggren *et al.*, *Nature* 346:476, 1990; Schumacher *et al.*, *Cell* 62:563, 1990; Townsend *et al.*, *Cell* 62:285, 1990; Parker *et al.*, *J. Immunol.* 149:1896, 1992).

As used herein, "high affinity" with respect to HLA class I molecules is defined as binding with an IC_{50}, or K_D value, of 50 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 50 and about 500 nM. "High affinity" with respect to binding to HLA class II molecules is defined as binding with an IC_{50} or K_D value of 100 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 100 and about 1000 nM.

The terms "identical" or percent "identity," in the context of two or more peptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithm or by manual alignment and visual inspection.

An "immunogenic peptide" or "peptide epitope" is a peptide that comprises an allele-specific motif or supermotif such that the peptide will bind an HLA molecule and induce a CTL and/or HTL response. Thus, immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and thereafter inducing an HLA-restricted cytotoxic or helper T cell response to the antigen from which the immunogenic peptide is derived.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment.

"Link" or "join" refers to any method known in the art for functionally connecting peptides, including, without limitation, recombinant fusion, covalent bonding, disulfide bonding, ionic bonding, hydrogen bonding, and electrostatic bonding.

"Major Histocompatibility Complex" or "MHC" is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, see, Paul, FUNDAMENTAL IMMUNOLOGY, 3RD ED., Raven Press, New York, 1993.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids, often 8 to 11 amino acids, for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

A "negative binding residue" or "deleterious residue" is an amino acid which, if present at certain positions (typically not primary anchor positions) in a peptide epitope, results in decreased binding affinity of the peptide for the peptide's corresponding HLA molecule.

A "non-native" sequence or "construct" refers to a sequence that is not found in nature, *i.e.*, is "non-naturally occurring". Such sequences include, *e.g.*, peptides that are lipidated or otherwise modified, and polyepitopic compositions that contain epitopes that are not contiguous in a native protein sequence.

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α-amino and carboxyl groups of adjacent amino acids. CTL-inducing peptides of the invention are often 13 residues or less in length and usually consist of between about 8 and about 11 residues, preferably 9 or 10 residues. HTL-inducing oligopeptides are often less than about 50 residues in length and usually consist of between about 6 and about 30 residues, more usually between about 12 and 25, and often between about 15 and 20 residues.

"Pharmaceutically acceptable" refers to a generally non-toxic, inert, and/or physiologically compatible composition.

A "pharmaceutical excipient" comprises a material such as an adjuvant, a carrier, pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservative, and the like.

A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three, usually two, primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. In one embodiment, for example, the primary anchor residues are located at position 2 (from the amino terminal position) and at the carboxyl terminal position of a 9-residue peptide epitope in accordance with the invention. The primary anchor positions for each motif and supermotif are set forth in Table I. For example, analog peptides can be created by altering the presence or absence of

particular residues in these primary anchor positions. Such analogs are used to modulate the binding affinity of a peptide comprising a particular motif or supermotif.

"Promiscuous recognition" is where a distinct peptide is recognized by the same T cell clone in the context of various HLA molecules. Promiscuous recognition or binding is synonymous with cross-reactive binding.

A "protective immune response" or "therapeutic immune response" refers to a CTL and/or an HTL response to an antigen derived from an infectious agent or a tumor antigen, which prevents or at least partially arrests disease symptoms or progression. The immune response may also include an antibody response which has been facilitated by the stimulation of helper T cells.

The term "residue" refers to an amino acid or amino acid mimetic incorporated into an oligopeptide by an amide bond or amide bond mimetic.

A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at "secondary anchor positions." A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or intermediate affinity binding peptides, or a residue otherwise associated with high or intermediate affinity binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A "subdominant epitope" is an epitope which evokes little or no response upon immunization with whole antigens which comprise the epitope, but for which a response can be obtained by immunization with an isolated peptide, and this response (unlike the case of cryptic epitopes) is detected when whole protein is used to recall the response *in vitro* or *in vivo*.

A "supermotif" is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. Preferably, a supermotif-bearing peptide is recognized with high or intermediate affinity (as defined herein) by two or more HLA molecules.

"Synthetic peptide" refers to a peptide that is man-made using such methods as chemical synthesis or recombinant DNA technology.

As used herein, a "vaccine" is a composition that contains one or more peptides of the invention. There are numerous embodiments of vaccines in accordance with the invention, such as by a cocktail of one or more peptides; one or more epitopes of the invention comprised by a polyepitopic peptide; or nucleic acids that encode such peptides or polypeptides, *e.g.*, a minigene that encodes a polyepitopic peptide. The "one or more peptides" can include any whole unit integer from 1-150, *e.g.*, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 , 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 or more peptides of the invention. The peptides or polypeptides can optionally be modified, such as by lipidation, addition of targeting or other sequences. HLA class I-binding peptides of the invention can be admixed with, or linked to, HLA class II-binding peptides, to facilitate activation of

both cytotoxic T lymphocytes and helper T lymphocytes. Vaccines can also comprise peptide-pulsed antigen presenting cells, *e.g.*, dendritic cells.

The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino to carboxyl direction with position one being the position closest to the amino terminal end of the epitope, or the peptide or protein of which it may be a part. In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G. The amino acid sequences of peptides set forth herein are generally designated using the standard single letter symbol. (A, Alanine; C, Cysteine; D, Aspartic Acid; E, Glutamic Acid; F, Phenylalanine; G, Glycine; H, Histidine; I, Isoleucine; K, Lysine; L, Leucine; M, Methionine; N, Asparagine; P, Proline; Q, Glutamine; R, Arginine; S, Serine; T, Threonine; V, Valine; W, Tryptophan; and Y, Tyrosine.) In addition to these symbols, "B"in the single letter abbreviations used herein designates α-amino butyric acid.

IV.B. Stimulation of CTL and HTL responses

The mechanism by which T cells recognize antigens has been delineated during the past ten years. Based on our understanding of the immune system we have developed efficacious peptide epitope vaccine compositions that can induce a therapeutic or prophylactic immune response to a TAA in a broad population. For an understanding of the value and efficacy of the claimed compositions, a brief review of immunology-related technology is provided.

A complex of an HLA molecule and a peptidic antigen acts as the ligand recognized by HLA-restricted T cells (Buus, S. *et al.*, *Cell* 47:1071, 1986; Babbitt, B. P. *et al.*, *Nature* 317:359, 1985; Townsend, A. and Bodmer, H., *Annu. Rev. Immunol.* 7:601, 1989; Germain, R. N., *Annu. Rev. Immunol.* 11:403, 1993). Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues that correspond to motifs required for specific binding to HLA antigen molecules have been identified and are described herein and are set forth in Tables I, II, and III (*see* also, *e.g.*, Southwood, *et al.*, *J. Immunol.* 160:3363, 1998; Rammensee, *et al.*, *Immunogenetics* 41:178, 1995; Rammensee *et al.*, SYFPEITHI, access via web at : http://134.2.96.221/scripts.hlaserver.dll/home.htm; Sette, A. and Sidney, J. *Curr. Opin. Immunol.* 10:478, 1998; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994; Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992; Sinigaglia, F. and Hammer, J. *Curr. Biol.* 6:52, 1994; Ruppert *et al.*, *Cell* 74:929-937, 1993; Kondo *et al.*, *J. Immunol.* 155:4307-4312, 1995; Sidney *et al.*, *J. Immunol.* 157:3480-3490, 1996; Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; Sette, A. and Sidney, J. *Immunogenetics 1999 Nov;50(3-4):201-12,* Review 9).

Furthermore, x-ray crystallographic analysis of HLA-peptide complexes has revealed pockets within the peptide binding cleft of HLA molecules which accommodate, in an allele-specific mode, residues borne by peptide ligands; these residues in turn determine the HLA binding capacity of the peptides in which they are present. (*See, e.g.*, Madden, D.R. *Annu. Rev. Immunol.* 13:587, 1995; Smith, *et al.*, *Immunity* 4:203, 1996; Fremont *et al.*, *Immunity* 8:305, 1998; Stern *et al.*, *Structure* 2:245, 1994; Jones, E.Y. *Curr. Opin. Immunol.* 9:75, 1997; Brown, J. H. *et al.*, *Nature* 364:33, 1993; Guo, H. C. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:8053, 1993; Guo, H. C. *et al.*, *Nature* 360:364, 1992; Silver, M. L. *et al.*, *Nature* 360:367, 1992; Matsumura, M. *et al.*, *Science* 257:927, 1992; Madden *et al.*, *Cell* 70:1035, 1992; Fremont, D. H. *et al.*, *Science* 257:919, 1992; Saper, M. A. , Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991.)

Accordingly, the definition of class I and class II allele-specific HLA binding motifs, or class I or class II supermotifs allows identification of regions within a protein that have the potential of binding particular HLA molecules.

The present inventors have found that the correlation of binding affinity with immunogenicity, which is disclosed herein, is an important factor to be considered when evaluating candidate peptides. Thus, by a combination of motif searches and HLA-peptide binding assays, candidates for epitope-based vaccines have been identified. After determining their binding affinity, additional confirmatory work can be performed to select, amongst these vaccine candidates, epitopes with preferred characteristics in terms of population coverage, antigenicity, and immunogenicity.

Various strategies can be utilized to evaluate immunogenicity, including:

1) Evaluation of primary T cell cultures from normal individuals (*see, e.g.*, Wentworth, P. A. *et al.*, *Mol. Immunol.* 32:603, 1995; Celis, E. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:2105, 1994; Tsai, V. *et al.*, *J. Immunol.* 158:1796, 1997; Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998); This procedure involves the stimulation of peripheral blood lymphocytes (PBL) from normal subjects with a test peptide in the presence of antigen presenting cells *in vitro* over a period of several weeks. T cells specific for the peptide become activated during this time and are detected using, *e.g.*, a lymphokine-release or a ^{51}Cr cytotoxicity assay involving peptide sensitized target cells.

2) Immunization of HLA transgenic mice (*see, e.g.*, Wentworth, P. A. *et al.*, *J. Immunol.* 26:97, 1996; Wentworth, P. A. *et al.*, *Int. Immunol.* 8:651, 1996; Alexander, J. *et al.*, *J. Immunol.* 159:4753, 1997); In this method, peptides in incomplete Freund's adjuvant are administered subcutaneously to HLA transgenic mice. Several weeks following immunization, splenocytes are removed and cultured *in vitro* in the presence of test peptide for approximately one week. Peptide-specific T cells are detected using, *e.g.*, a ^{51}Cr-release assay involving peptide sensitized target cells and target cells expressing endogenously generated antigen.

3) Demonstration of recall T cell responses from patients who have been effectively vaccinated or who have a tumor; (*see, e.g.*, Rehermann, B. *et al.*, *J. Exp. Med.* 181:1047, 1995; Doolan, D. L. *et al.*, *Immunity* 7:97, 1997; Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; Diepolder, H. M. *et al.*, *J. Virol.* 71:6011, 1997; Tsang *et al.*, *J. Natl. Cancer Inst.* 87:982-990, 1995; Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996). In applying this strategy, recall responses are detected by culturing PBL from patients with cancer who have generated an immune response

"naturally", or from patients who were vaccinated with tumor antigen vaccines. PBL from subjects are cultured *in vitro* for 1-2 weeks in the presence of test peptide plus antigen presenting cells (APC) to allow activation of "memory" T cells, as compared to "naive" T cells. At the end of the culture period, T cell activity is detected using assays for T cell activity including ^{51}Cr release involving peptide-sensitized targets, T cell proliferation, or lymphokine release.

The following describes the peptide epitopes and corresponding nucleic acids of the invention.

IV.C. Binding Affinity of Peptide Epitopes for HLA Molecules

As indicated herein, the large degree of HLA polymorphism is an important factor to be taken into account with the epitope-based approach to vaccine development. To address this factor, epitope selection encompassing identification of peptides capable of binding at high or intermediate affinity to multiple HLA molecules is preferably utilized, most preferably these epitopes bind at high or intermediate affinity to two or more allele-specific HLA molecules.

CTL-inducing peptides of interest for vaccine compositions preferably include those that have an IC_{50} or binding affinity value for class I HLA molecules of 500 nM or better (*i.e.*, the value is ≤ 500 nM). HTL-inducing peptides preferably include those that have an IC_{50} or binding affinity value for class II HLA molecules of 1000 nM or better, (*i.e.*, the value is ≤ 1,000 nM). For example, peptide binding is assessed by testing the capacity of a candidate peptide to bind to a purified HLA molecule *in vitro*. Peptides exhibiting high or intermediate affinity are then considered for further analysis. Selected peptides are tested on other members of the supertype family. In preferred embodiments, peptides that exhibit cross-reactive binding are then used in cellular screening analyses or vaccines.

High HLA binding affinity is correlated with greater immunogenicity (*see, e.g.*, Sette, *et al., J. Immunol.* 153:5586-5592, 1994; Chen *et al., J. Immunol.* 152:2874-2881, 1994; and Ressing *et al., J. Immunol.* 154:5934-5943, 1995). Greater immunogenicity can be manifested in several different ways. Immunogenicity corresponds to whether an immune response is elicited at all, and to the vigor of any particular response, as well as to the extent of a population in which a response is elicited. For example, a peptide might elicit an immune response in a diverse array of the population, yet in no instance produce a vigorous response. Moreover, higher binding affinity peptides lead to more vigorous immunogenic responses. As a result, less peptide is required to elicit a similar biological effect if a high or intermediate affinity binding peptide is used. Thus, in preferred embodiments of the invention, high or intermediate affinity binding epitopes are particularly useful.

The relationship between binding affinity for HLA class I molecules and immunogenicity of discrete peptide epitopes on bound antigens has been determined for the first time in the art by the present inventors. The correlation between binding affinity and immunogenicity was analyzed in two different experimental approaches (*see, e.g.*, Sette, *et al., J. Immunol.* 153:5586-5592, 1994). In the first approach, the immunogenicity of potential epitopes ranging in HLA binding affinity over a 10,000-fold range was analyzed in HLA-A*0201 transgenic mice. In the second approach, the antigenicity of approximately 100 different hepatitis B virus (HBV)-derived potential epitopes, all carrying A*0201 binding motifs, was assessed by using PBL from acute hepatitis patients. Pursuant to these approaches, it

was determined that an affinity threshold value of approximately 500 nM (preferably 50 nM or less) determines the capacity of a peptide epitope to elicit a CTL response. These data are true for class I binding affinity measurements for naturally processed peptides and for synthesized T cell epitopes. These data also indicate the important role of determinant selection in the shaping of T cell responses (*see, e.g.*, Schaeffer *et al., Proc. Natl. Acad. Sci. USA* 86:4649-4653, 1989).

An affinity threshold associated with immunogenicity in the context of HLA class II DR molecules has also been delineated (*see, e.g.*, Southwood *et al. J. Immunology* 160:3363-3373,1998, and co-pending U.S.S.N. 09/009,953 filed 1/21/98). In order to define a biologically significant threshold of DR binding affinity, a database of the binding affinities of 32 DR-restricted epitopes for their restricting element (*i.e.*, the HLA molecule that binds the motif) was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, *i.e.* binding affinity values of 100 nM or less. In the other half of the cases (16 of 32), DR restriction was associated with intermediate affinity (binding affinity values in the 100-1000 nM range). In only one of 32 cases was DR restriction associated with an IC_{50} of 1000 nM or greater. Thus, 1000 nM can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

In the case of tumor-associated antigens, many CTL peptide epitopes that have been shown to induce CTL that lyse peptide-pulsed target cells and tumor cell targets endogenously expressing the epitope exhibit binding affinity or IC_{50} values of 200 nM or less. In a study that evaluated the association of binding affinity and immunogenicity of a small set of such TAA epitopes, 100% (10/10) of the high binders, *i.e.*, peptide epitopes binding at an affinity of 50 nM or less, were immunogenic and 80% (8/10) of them elicited CTLs that specifically recognized tumor cells. In the 51 to 200 nM range, very similar figures were obtained. With respect to analog peptides, CTL inductions positive for wildtype peptide and tumor cells were noted for 86% (6/7) and 71% (5/7) of the peptides, respectively. In the 201-500 nM range, most peptides (4/5 wildtype) were positive for induction of CTL recognizing wildtype peptide, but tumor recognition was not detected.

The binding affinity of peptides for HLA molecules can be determined as described in Example 1, below.

IV.D. Peptide Epitope Binding Motifs and Supermotifs

Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues required for allele-specific binding to HLA molecules have been identified. The presence of these residues correlates with binding affinity for HLA molecules. The identification of motifs and/or supermotifs that correlate with high and intermediate affinity binding is an important issue with respect to the identification of immunogenic peptide epitopes for the inclusion in a vaccine. Kast *et al.* (*J. Immunol.* 152:3904-3912, 1994) have shown that motif-bearing peptides account for 90% of the epitopes that bind to allele-specific HLA class I molecules. In this study all possible peptides of 9 amino acids in length and overlapping by eight amino acids (240 peptides), which cover the entire sequence of the E6 and E7 proteins of human papillomavirus type 16, were evaluated for binding to five allele-specific HLA molecules that are expressed at high frequency among different ethnic groups. This unbiased set of peptides allowed an evaluation of the predictive value of HLA class I motifs.

From the set of 240 peptides, 22 peptides were identified that bound to an allele-specific HLA molecule with high or intermediate affinity. Of these 22 peptides, 20 (*i.e.* 91%) were motif-bearing. Thus, this study demonstrates the value of motifs for the identification of peptide epitopes for inclusion in a vaccine: application of motif-based identification techniques will identify about 90% of the potential epitopes in a target antigen protein sequence.

Such peptide epitopes are identified in the Tables described below.

Peptides of the present invention may also comprise epitopes that bind to MHC class II DR molecules. A greater degree of heterogeneity in both size and binding frame position of the motif, relative to the N and C termini of the peptide, exists for class II peptide ligands. This increased heterogeneity of HLA class II peptide ligands is due to the structure of the binding groove of the HLA class II molecule which, unlike its class I counterpart, is open at both ends. Crystallographic analysis of HLA class II DRB*0101-peptide complexes showed that the major energy of binding is contributed by peptide residues complexed with complementary pockets on the DRB*0101 molecules. An important anchor residue engages the deepest hydrophobic pocket (*see, e.g.*, Madden, D.R. *Ann. Rev. Immunol.* 13:587, 1995) and is referred to as position 1 (P1). P1 may represent the N-terminal residue of a class II binding peptide epitope, but more typically is flanked towards the N-terminus by one or more residues. Other studies have also pointed to an important role for the peptide residue in the 6th position towards the C-terminus, relative to P1, for binding to various DR molecules.

In the past few years evidence has accumulated to demonstrate that a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets. Thus, peptides of the present invention are identified by any one of several HLA-specific amino acid motifs (*see, e.g.*, Tables I-III), or if the presence of the motif corresponds to the ability to bind several allele-specific HLA molecules, a supermotif. The HLA molecules that bind to peptides that possess a particular amino acid supermotif are collectively referred to as an HLA "supertype."

The peptide motifs and supermotifs described below, and summarized in Tables I-III, provide guidance for the identification and use of peptide epitopes in accordance with the invention.

Examples of supermotif and/or motif-bearing peptide epitopes are shown in Tables VII-XX. To obtain the peptide epitope sequences, protein sequence data for the prostate cancer antigens PAP, PSA, PSM, and hK2, which is designated as kallikrein in Tables VII-XX, were evaluated for the presence of the designated supermotif or motif. The "Position" column indicates the position in the protein sequence that corresponds to the first amino acid residue of the putative epitope. The "number of amino acids" indicates the number of residues in the epitope sequence. The tables also include a binding affinity ratio listing for some of the peptide epitopes for the allele-specific HLA molecule indicated in the column heading. The ratio may be converted to IC_{50} by using the following formula: IC_{50} of the standard peptide/ratio = IC_{50} of the test peptide (*i.e.*, the peptide epitope). The IC_{50} values of standard peptides used to determine binding affinities for Class I peptides are shown in Table IV. The IC_{50} values of standard peptides used to determine binding affinities for Class II peptides are shown in Table V. The peptides used as standards for the binding assays described herein are examples of standards; alternative standard peptides can also be used when performing binding studies.

To obtain the peptide epitope sequences listed in each of Tables VII-XX, the amino acid sequences of PSA, PSM, PAP, and HuK were evaluated for the presence of the designated supermotif or motif, *i.e.*, the amino acid sequence was searched for the presence of the primary anchor residues as set out in Table I (for Class I motifs) or Table III (for Class II motifs) for each respective motif or supermotif.

In the Tables, the motif- and/or supermotif-bearing amino acid sequences are identified by the position number and the length of the epitope with reference to the prostate antigen amino acid sequence and numbering provided below. The "protein" indicates the prostate antigen sequence that includes the epitope. The "pos" (position) column designates the amino acid position in the prostate antigen sequence protein sequence below that corresponds to the first amino acid residue of the epitope. The "number of amino acids" indicates the number of residues in the epitope sequence and hence, the length of the epitope. For example, the first peptide sequence listed in Table VII is a sequence of 11 residues in length starting at position 122 of PAP. Accordingly, the amino acid sequence of the epitope is ALFPPEGVSIW. Similarly, the first kallikrein sequence in Table VII starts at position147 and is 11 residues in length. Thus the amino acid sequence is ALGTTCYASGW.

Binding data presented in Tables VII-XX are expressed as a relative binding ratio, *supra* in the in columns labeled with the allele-specific HLA molecule.

PSA (Prostate Specific Antigen)

```
1 VVFLTLSVTW IGAAPLILSR IVGGWECEKH SQPWQVLVAS RGRAVCGGVL VHPQWVLTAA   60
  HCIRNKSVIL LGRHSLFHPE DTGQVFQVSH SFPHPLYDMS LLKNRFLRPG DDSSHDLMLL  120
  RLSEPAELTD AVKVMDLPTQ EPALGTTCYA SGWGSIEPEE FLTPKKLQCV DLHVISNDVC  180
  AQVHPQKVTK FMLCAGRWTG GKSTCSGDSG GPLVCNGVLQ GITSWGSEPC ALPERPSLYT  240
  KVVHYRKWIK DTIVANP  257
```

PAP (Prostatic Acid Phosphatase)

```
1 MRAAPLLLAR AASLSLGFLF LLFFWLDRSV LAKELKFVTL VFRHGDRSPI DTFPTDPIKE   60
  SSWPQGFGQL TQLGMEQHYE LGEYIRKRYR KFLNESYKHE QVYIRSTDVD RTLMSAMTNL  120
  AALFPPEGVS IWNPILLWQP IPVHTVPLSE DQLLYLPFRN CPRFQELESE TLKSEEFQKR  180
  LHPYKDFIAT LGKLSGLHGQ DLFGIWSKVY DPLYCESVHN FTLPSWATED TMTKLRELSE  240
  LSLLSLYGIH KQKEKSRLQG GVLVNEILNH MKRATQIPSY KKLIMYSAHD TTVSGLQMAL  300
  DVYNGLLPPY ASCHLTELYF EKGEYFVEMY YRNETQHEPY PLMLPGCSPS CPLERFAELV  360
  GPVIPQDWST ECMTTNSHQG TEDSTD  386
```

PSM (prostate specific membrane antigen)

```
1 MWNLLHETDS AVATARRPRW LCAGALVLAG GFFLLGFLFG WFIKSSNEAT NITPKHNMKA   60
  FLDELKAENI KKFLYNFTQI PHLAGTEQNF QLAKQIQSQW KEFGLDSVEL AHYDVLLSYP  120
  NKTHPNYISI INEDGNEIFN TSLFEPPPPG YENVSDIVPP FSAFSPQGMP EGDLVYVNYA  180
  RTEDFFKLER DMKINCSGKI VIARYGKVFR GNKVKNAQLA GAKGVILYSD PADYFAPGVK  240
  SYPDGWNLPG GGVQRGNILN LNGAGDPLTP GYPANEYAYR RGIAEAVGLP SIPVHPIGYY  300
  DAQKLLEKMG GSAPPDSSWR GSLKVPYNVG PGFTGNFSTQ KVKMHIHSTN EVTRIYNVIG  360
  TLRGAVEPDR YVILGGHRDS WVFGGIDPQS GAAVVHEIVR SFGTLKKEGW RPRRTILFAS  420
```

WDAEEPGLLG STEWAEENSR LLQERGVAYI NADSSIEGNY TLRVDCTPLM YSLVHNLTKE 480
LKSPDEGFEG KSLYESWTKK SPSPEFSGMP RISKLGSGND FEVFFQRLGI ASGRARYTKN 540
WETNKFSGYP LYHSVYETYE LVEKFYDPMF KYHLTVAQVR GGMVFELANS IVLPFDCRDY 600
AVVLRKYADK IYSISMKHPQ EMKTYSVSFD SLFSAVKNFT EIASKFSERL QDFDKSNPIV 660
LRMMNDQLMF LERAFIDPLG LPDRPFYRHV IYAPSSHNKY AGESFPGIYD ALFDIESKVD 720
PSKAWGEVKR QIYVAAFTVQ AAAETLSEVA 750

Kallikrein (human kallikrein2, Accession NM005551)

MWDLVLSIAL SVGCTGAVPL IQSRIVGGWE CEKHSQPWQV AVYSHGWAHC GGVLVHPQWV 60
LTAAHCLKKN SQVWLGRHNL FEPEDTGQRV PVSHSFPHPL YNMSLLKHQS LRPDEDSSHD 120
LMLLRLSEPA KITDVVKVLG LPTQEPALGT TCYASGWGSI EPEEFLRPRS LQCVSLHLLS 180
NDMCARAYSE KVTEFMLCAG LWTGGKDTCG GDSGGPLVCN GVLQGITSWG PEPCALPEKP 240
AVYTKVVHYR KWIKDTIAAN P 261

HLA Class I Motifs Indicative of CTL Inducing Peptide Epitopes:

The primary anchor residues of the HLA class I peptide epitope supermotifs and motifs delineated below are summarized in Table I. The HLA class I motifs set out in Table I(a) are those most particularly relevant to the invention claimed here. Primary and secondary anchor positions are summarized in Table II. Allele-specific HLA molecules that comprise HLA class I supertype families are listed in Table VI. In some cases, peptide epitopes may be listed in both a motif and a supermotif Table. The relationship of a particular motif and respective supermotif is indicated in the description of the individual motifs.

IV.D.1. HLA-A1 supermotif

The HLA-A1 supermotif is characterized by the presence in peptide ligands of a small (T or S) or hydrophobic (L, I, V, or M) primary anchor residue in position 2, and an aromatic (Y, F, or W) primary anchor residue at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind to the A1 supermotif (*i.e.*, the HLA-A1 supertype) is comprised of at least: A*0101, A*2601, A*2602, A*2501, and A*3201 (*see, e.g.*, DiBrino, M. *et al.*, *J. Immunol.* 151:5930, 1993; DiBrino, M. *et al.*, *J. Immunol.* 152:620, 1994; Kondo, A. *et al.*, *Immunogenetics* 45:249, 1997). Other allele-specific HLA molecules predicted to be members of the A1 superfamily are shown in Table VI. Peptides binding to each of the individual HLA proteins can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise an A1 supermotif are set forth on the attached Table VII.

IV.D.2. HLA-A2 supermotif

Primary anchor specificities for allele-specific HLA-A2.1 molecules (*see, e.g.*, Falk *et al.*, *Nature* 351:290-296, 1991; Hunt *et al.*, *Science* 255:1261-1263, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992; Ruppert *et al.*, *Cell* 74:929-937, 1993) and cross-reactive binding among HLA-A2 and -A28

molecules have been described. (*See, e.g.*, Fruci *et al.*, *Human Immunol.* 38:187-192, 1993; Tanigaki *et al.*, *Human Immunol.* 39:155-162, 1994; Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994 for reviews of relevant data.) These primary anchor residues define the HLA-A2 supermotif; which presence in peptide ligands corresponds to the ability to bind several different HLA-A2 and -A28 molecules. The HLA-A2 supermotif comprises peptide ligands with L, I, V, M, A, T, or Q as a primary anchor residue at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope.

The corresponding family of HLA molecules (*i.e.*, the HLA-A2 supertype that binds these peptides) is comprised of at least: A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, and A*6901. Other allele-specific HLA molecules predicted to be members of the A2 superfamily are shown in Table VI. As explained in detail below, binding to each of the individual allele-specific HLA molecules can be modulated by substitutions at the primary anchor and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise an A2 supermotif are set forth on the attached Table VIII. The motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.3. HLA-A3 supermotif

The HLA-A3 supermotif is characterized by the presence in peptide ligands of A, L, I, V, M, S, or, T as a primary anchor at position 2, and a positively charged residue, R or K, at the C-terminal position of the epitope, *e.g.*, in position 9 of 9-mers (*see, e.g.*, Sidney *et al.*, *Hum. Immunol.* 45:79, 1996). Exemplary members of the corresponding family of HLA molecules (the HLA-A3 supertype) that bind the A3 supermotif include at least: A*0301, A*1101, A*3101, A*3301, and A*6801. Other allele-specific HLA molecules predicted to be members of the A3 supertype are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions of amino acids at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A3 supermotif are set forth on the attached Table IX.

IV.D.4. HLA-A24 supermotif

The HLA-A24 supermotif is characterized by the presence in peptide ligands of an aromatic (F, W, or Y) or hydrophobic aliphatic (L, I, V, M, or T) residue as a primary anchor in position 2, and Y, F, W, L, I, or M as primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sette and Sidney, *Immunogenetics 1999 Nov;50(3-4):201-12,* Review). The corresponding family of HLA molecules that bind to the A24 supermotif (*i.e.*, the A24 supertype) includes at least: A*2402, A*3001, and A*2301. Other allele-specific HLA molecules predicted to be members of the A24 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A24 supermotif are set forth on the attached Table X.

IV.D.5. HLA-B7 supermotif

The HLA-B7 supermotif is characterized by peptides bearing proline in position 2 as a primary anchor, and a hydrophobic or aliphatic amino acid (L, I, V, M, A, F, W, or Y) as the primary anchor at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind the B7 supermotif (*i.e.*, the HLA-B7 supertype) is comprised of at least twenty six HLA-B proteins comprising at least: B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, and B*7801 (*see, e.g.*, Sidney, *et al., J. Immunol.* 154:247, 1995; Barber, *et al., Curr. Biol.* 5:179, 1995; Hill, *et al., Nature* 360:434, 1992; Rammensee, *et al., Immunogenetics* 41:178, 1995 for reviews of relevant data). Other allele-specific HLA molecules predicted to be members of the B7 supertype are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B7 supermotif are set forth on the attached Table XI.

IV.D.6. HLA-B27 supermotif

The HLA-B27 supermotif is characterized by the presence in peptide ligands of a positively charged (R, H, or K) residue as a primary anchor at position 2, and a hydrophobic (F, Y, L, W, M, I, A, or V) residue as a primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics 1999 Nov;50(3-4):201-12,* Review). Exemplary members of the corresponding family of HLA molecules that bind to the B27 supermotif (*i.e.*, the B27 supertype) include at least B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, and B*7301. Other allele-specific HLA molecules predicted to be members of the B27 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B27 supermotif are set forth on the attached Table XII.

IV.D.7. HLA-B44 supermotif

The HLA-B44 supermotif is characterized by the presence in peptide ligands of negatively charged (D or E) residues as a primary anchor in position 2, and hydrophobic residues (F, W, Y, L, I, M, V, or A) as a primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sidney et al., *Immunol. Today* 17:261, 1996). Exemplary members of the corresponding family of HLA molecules that bind to the B44 supermotif (*i.e.*, the B44 supertype) include at least: B*1801, B*1802, B*3701, B*4001, B*4002, B*4006, B*4402, B*4403, and B*4404. Peptide binding to each of the allele-specific HLA

molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the supermotif.

IV.D.8. HLA-B58 supermotif

The HLA-B58 supermotif is characterized by the presence in peptide ligands of a small aliphatic residue (A, S, or T) as a primary anchor residue at position 2, and an aromatic or hydrophobic residue (F, W, Y, L, I, V, M, or A) as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics 1999 Nov;50(3-4):201-12,* Review). Exemplary members of the corresponding family of HLA molecules that bind to the B58 supermotif (*i.e.*, the B58 supertype) include at least: B*1516, B*1517, B*5701, B*5702, and B*5801. Other allele-specific HLA molecules predicted to be members of the B58 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B27 supermotif are set forth on the attached Table XII.

IV.D.9. HLA-B62 supermotif

The HLA-B62 supermotif is characterized by the presence in peptide ligands of the polar aliphatic residue Q or a hydrophobic aliphatic residue (L, V, M, I, or P) as a primary anchor in position 2, and a hydrophobic residue (F, W, Y, M, I, V, L, or A) as a primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics 1999 Nov;50(3-4):201-12,* Review). Exemplary members of the corresponding family of HLA molecules that bind to the B62 supermotif (*i.e.*, the B62 supertype) include at least: B*1501, B*1502, B*1513, and B5201. Other allele-specific HLA molecules predicted to be members of the B62 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B62 supermotif are set forth on the attached Table XIV.

IV.D.10. HLA-A1 motif

The HLA-A1 motif is characterized by the presence in peptide ligands of T, S, or M as a primary anchor residue at position 2 and the presence of Y as a primary anchor residue at the C-terminal position of the epitope. An alternative allele-specific A1 motif is characterized by a primary anchor residue at position 3 rather than position 2. This motif is characterized by the presence of D, E, A, or S as a primary anchor residue in position 3, and a Y as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, DiBrino *et al.*, J. Immunol., 152:620, 1994; Kondo *et al.*, *Immunogenetics* 45:249, 1997; and Kubo *et al.*, *J. Immunol.* 152:3913, 1994 for reviews of relevant data). Peptide binding to HLA-A1 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise either A1 motif are set forth on the attached Table XV. Those epitopes comprising T, S, or M at position 2 and Y at the C-terminal position are also included in the listing of HLA-A1 supermotif-bearing peptide epitopes listed in Table VII, as these residues are a subset of the A1 supermotif.

IV.D.11. HLA-A*0201 motif

An HLA-A2*0201 motif was determined to be characterized by the presence in peptide ligands of L or M as a primary anchor residue in position 2, and L or V as a primary anchor residue at the C-terminal position of a 9-residue peptide (*see, e.g.*, Falk *et al.*, *Nature* 351:290-296, 1991) and was further found to comprise an I at position 2 and I or A at the C-terminal position of a nine amino acid peptide (*see, e.g.*, Hunt *et al.*, Science 255:1261-1263, March 6, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992). The A*0201 allele-specific motif has also been defined by the present inventors to additionally comprise V, A, T, or Q as a primary anchor residue at position 2, and M or T as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994). Thus, the HLA-A*0201 motif comprises peptide ligands with L, I, V, M, A, T, or Q as primary anchor residues at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope. The preferred and tolerated residues that characterize the primary anchor positions of the HLA-A*0201 motif are identical to the residues describing the A2 supermotif. (For reviews of relevant data, *see, e.g.*, del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Ruppert *et al.*, *Cell* 74:929-937, 1993; Sidney *et al.*, *Immunol. Today* 17:261-266, 1996; Sette and Sidney, *Curr. Opin. in Immunol.* 10:478-482, 1998). Secondary anchor residues that characterize the A*0201 motif have additionally been defined (*see, e.g.*, Ruppert *et al.*, *Cell* 74:929-937, 1993). These are shown in Table II. Peptide binding to HLA-A*0201 molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise an A*0201 motif are set forth on the attached Table VII. The A*0201 motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.12. HLA-A3 motif

The HLA-A3 motif is characterized by the presence in peptide ligands of L, M, V, I, S, A, T, F, C, G, or D as a primary anchor residue at position 2, and the presence of K, Y, R, H, F, or A as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, DiBrino *et al.*, *Proc. Natl. Acad. Sci USA* 90:1508, 1993; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A3 motif are set forth on the attached Table XVI. Those epitopes that comprise the A3 supermotif are also listed in Table IX, as the A3 supermotif primary anchor residues comprise a subset of the A3- and A11-allele-specific motifs.

IV.D.13. HLA-A11 motif

The HLA-A11 motif is characterized by the presence in peptide ligands of V, T, M, L, I, S, A, G, N, C, D, or F as a primary anchor residue in position 2, and K, R, Y, or H as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, Zhang *et al.*, *Proc. Natl. Acad. Sci USA* 90:2217-2221, 1993; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A11 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A11 motif are set forth on the attached Table XVII; peptide epitopes comprising the A3 allele-specific motif are also present in this Table because of the extensive overlap between the A3 and A11 motif primary anchor specificities. Further, those peptide epitopes that comprise the A3 supermotif are also listed in Table IX.

IV.D.14. HLA-A24 motif

The HLA-A24 motif is characterized by the presence in peptide ligands of Y, F, W, or M as a primary anchor residue in position 2, and F, L, I, or W as a primary anchor residue at the C-terminal position of the epitope (*see, e.g., Kondo et al., J. Immunol.* 155:4307-4312, 1995; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A24 molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A24 motif are set forth on the attached Table XVIII. These epitopes are also listed in Table X, which sets forth HLA-A24-supermotif-bearing peptide epitopes, as the primary anchor residues characterizing the A24 allele-specific motif comprise a subset of the A24 supermotif primary anchor residues.

Motifs Indicative of Class II HTL Inducing Peptide Epitopes

The primary and secondary anchor residues of the HLA class II peptide epitope supermotifs and motifs delineated below are summarized in Table III.

IV.D.15. HLA DR-1-4-7 supermotif

Motifs have also been identified for peptides that bind to three common HLA class II allele-specific HLA molecules: HLA DRB1*0401, DRB1*0101, and DRB1*0701 (*see, e.g.*, the review by Southwood *et al. J. Immunology* 160:3363-3373,1998). Collectively, the common residues from these motifs delineate the HLA DR-1-4-7 supermotif. Peptides that bind to these DR molecules carry a supermotif characterized by a large aromatic or hydrophobic residue (Y, F, W, L, I, V, or M) as a primary anchor residue in position 1, and a small, non-charged residue (S, T, C, A, P, V, I, L, or M) as a primary anchor residue in position 6 of a 9-mer core region. Allele-specific secondary effects and secondary anchors for each of these HLA types have also been identified (Southwood *et al., supra*). These are set forth in Table III. Peptide binding to HLA- DRB1*0401, DRB1*0101, and/or DRB1*0701 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative 9-mer peptide sequences comprising the DR-1-4-7 supermotif, wherein position 1 of the supermotif is at position 1 of the nine-residue core, are set forth in Table XIX. For each sequence, the "protein" column indicates the prostate-associated antigen, *i.e.*, PSA, PSM, PAP, or HuK2 (kallikrein). The "position" column designates the amino acid position in the prostate antigen protein sequence that corresponds to the first amino acid residue of the core sequence. The core sequences are all 9 residues in length. For example, the first PSM sequence listed in Table XIX is a core sequence of nine residues in length that starts at position 611 of the PSM amino acid sequence provided herein. Accordingly, the amino acid sequence of the core sequence is IYSISMKHP. Exemplary epitopes of 15 amino acids in length that comprises the nine residue core include the three residues on either side that flank the nine residue core. For example, the exemplary epitope of 15 amino acids in length that comprises the core epitope at position 611 of PSM is ADKIYSISMKHPQEM.

HTL epitopes that comprise the core sequences can also be of lengths other than 15 amino acids, *supra*. For example, epitopes of the invention include sequences that comprise the nine residue core plus the 1, 2, 3 (as in the exemplary 15-mer), 4, or 5 flanking residues immediately adjacent to the nine residue core on each side.

IV.D.16. HLA-DR3 motifs

Two alternative motifs (*i.e.*, submotifs) characterize peptide epitopes that bind to HLA-DR3 molecules (*see, e.g.*, Geluk *et al.*, *J. Immunol.* 152:5742, 1994). In the first motif (submotif DR3a) a large, hydrophobic residue (L, I, V, M, F, or Y) is present in anchor position 1 of a 9-mer core, and D is present as an anchor at position 4, towards the carboxyl terminus of the epitope. As in other class II motifs, core position 1 may or may not occupy the peptide N-terminal position.

The alternative DR3 submotif provides for lack of the large, hydrophobic residue at anchor position 1, and/or lack of the negatively charged or amide-like anchor residue at position 4, by the presence of a positive charge at position 6 towards the carboxyl terminus of the epitope. Thus, for the alternative allele-specific DR3 motif (submotif DR3b): L, I, V, M, F, Y, A, or Y is present at anchor position 1; D, N, Q, E, S, or T is present at anchor position 4; and K, R, or H is present at anchor position 6. Peptide binding to HLA-DR3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Peptide epitope 9-mer core regions corresponding to a nine residue sequence comprising the DR3a or the DR3b submotifs (wherein position 1 of the motif is at position 1 of the nine residue core) are set forth in Table XXa and b. For each sequence, the "protein" column indicates the prostate-associated antigen, *i.e.*, PSA, PSM, PAP, or HuK2 (kallikrein). The "position" column designates the amino acid position in the prostate antigen protein sequence that corresponds to the first amino acid residue of the core sequence. The core sequences are all 9 residues in length. For example, the first sequence listed in Table XXa is a core sequence of nine residues in length that starts at position 124 of the PAP amino acid sequence provided herein. Accordingly, the amino acid sequence of the core sequence is FPPEGVSIW. Exemplary epitopes of 15 amino acids in length that comprises the nine residue core include the three residues on either side that flank the nine residue core. For example, the exemplary epitope of 15 amino acids in length that comprises the core epitope at position 124 of PAP is AALFPPEGVSIWNPI.

HTL epitopes that comprise the core sequences can also be of lengths other than 15 amino acids, *supra*. For example, epitopes of the invention include sequences that comprise the nine residue core plus the 1, 2, 3 (as in the exemplary 15-mer), 4, or 5 flanking residues immediately adjacent to the nine residue core on each side.

Each of the HLA class I or class II peptide epitopes identified as described herein is deemed singly to be an inventive aspect of this application. Further, it is also an inventive aspect of this application that each peptide epitope may be used in combination with any other peptide epitope.

IV.E. Enhancing Population Coverage of the Vaccine

Vaccines that have broad population coverage are preferred because they are more commercially viable and generally applicable to the most people. Broad population coverage can be obtained using the peptides of the invention (and/or nucleic acid compositions that encode such peptides) through selecting peptide epitopes that bind to HLA alleles which, when considered in total, are present in most of the population. Table XXI shows the overall frequencies of HLA class I supertypes in various ethnicities (Table XXIa) and the combined population coverage achieved by the A2-, A3-, and B7-supertypes (Table XXIb). The A2-, A3-, and B7 supertypes are each present on average of over 40% in each of these five major ethnic groups. Coverage in excess of 80% is achieved with a combination of these supermotifs. These results suggest that effective and non-ethnically biased population coverage is achieved upon use of a limited number of cross-reactive peptides. Although the population coverage reached with these three main peptide specificities is high, coverage can be expanded to reach 95% population coverage and above, and more easily achieve truly multispecific responses upon use of additional supermotif or allele-specific motif bearing peptides.

The B44-, A1-, and A24-supertypes are each present, on average, in a range from 25% to 40% in these major ethnic populations (Table XXIa). While less prevalent overall, the B27-, B58-, and B62 supertypes are each present with a frequency >25% in at least one major ethnic group (Table XXIa). Table XXIb summarizes the estimated prevalence of combinations of HLA supertypes that have been identified in five major ethnic groups; the incremental coverage obtained by the inclusion of A1,- A24-, and B44-supertypes to the A2, A3, and B7 coverage; and coverage obtained with all of the supertypes described herein, is shown.

The data presented herein, together with the previous definition of the A2-, A3-, and B7-supertypes, indicates that all antigens, with the possible exception of A29, B8, and B46, can be classified into a total of nine HLA supertypes. By including epitopes from the six most frequent supertypes, an average population coverage of 99% is obtained for five major ethnic groups.

IV.F. Immune Response-Stimulating Peptide Analogs

In general, CTL and HTL responses to whole antigens are not directed against all possible epitopes. Rather, they are restricted to a few "immunodominant" determinants (Zinkernagel, *et al., Adv. Immunol.* 27:5159, 1979; Bennink, *et al., J. Exp. Med.* 168:19351939, 1988; Rawle, *et al., J. Immunol.* 146:3977-3984, 1991). It has been recognized that immunodominance (Benacerraf, *et al., Science* 175:273-279, 1972) could be explained by either the ability of a given epitope to selectively bind a particular HLA

protein (determinant selection theory) (Vitiello, *et al., J. Immunol.* 131:1635, 1983); Rosenthal, *et al., Nature* 267:156-158, 1977), or to be selectively recognized by the existing TCR (T cell receptor) specificities (repertoire theory) (Klein, J., IMMUNOLOGY, THE SCIENCE OF SELF/NONSELF DISCRIMINATION, John Wiley & Sons, New York, pp. 270-310, 1982). It has been demonstrated that additional factors, mostly linked to processing events, can also play a key role in dictating, beyond strict immunogenicity, which of the many potential determinants will be presented as immunodominant (Sercarz, *et al., Annu. Rev. Immunol.* 11:729-766, 1993).

Because tissue specific and developmental TAAs are expressed on normal tissue at least at some point in time or location within the body, it may be expected that T cells to them, particularly dominant epitopes, are eliminated during immunological surveillance and that tolerance is induced. However, CTL responses to tumor epitopes in both normal donors and cancer patient have been detected, which may indicate that tolerance is incomplete (*see, e.g.*, Kawashima *et al.*, Hum. Immunol. 59:1, 1998; Tsang, *J. Natl. Cancer Inst.* 87:82-90, 1995; Rongcun *et al., J. Immunol.* 163:1037, 1999). Thus, immune tolerance does not completely eliminate or inactivate CTL precursors capable of recognizing high affinity HLA class I binding peptides.

An additional strategy to overcome tolerance is to use analog peptides. Without intending to be bound by theory, it is believed that because T cells to dominant epitopes may have been clonally deleted, selecting subdominant epitopes may allow existing T cells to be recruited, which will then lead to a therapeutic or prophylactic response. However, the binding of HLA molecules to subdominant epitopes is often less vigorous than to dominant ones. Accordingly, there is a need to be able to modulate the binding affinity of particular immunogenic epitopes for one or more HLA molecules, and thereby to modulate the immune response elicited by the peptide, for example to prepare analog peptides which elicit a more vigorous response.

Although peptides with suitable cross-reactivity among all alleles of a superfamily are identified by the screening procedures described above, cross-reactivity is not always as complete as possible, and in certain cases procedures to increase cross-reactivity of peptides can be useful; moreover, such procedures can also be used to modify other properties of the peptides such as binding affinity or peptide stability. Having established the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (*i.e.*, analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the broadest cross-reactivity patterns, can be produced in accordance with the teachings herein. The present concepts related to analog generation are set forth in greater detail in co-pending U.S.S.N. 09/226,775 filed 1/6/99.

In brief, the strategy employed utilizes the motifs or supermotifs which correlate with binding to certain HLA molecules. The motifs or supermotifs are defined by having primary anchors, and in many cases secondary anchors. Analog peptides can be created by substituting amino acid residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and motifs that have been defined for HLA class I and class II binding peptides are shown in Tables II and III, respectively.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or members of HLA supertypes that bind the respective motif or supermotif (Tables II and III). Accordingly, removal of such residues that are detrimental to binding can be performed in accordance with the present invention. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of peptides used in the analysis, the incidence of cross-reactivity increased from 22% to 37% (*see, e.g.*, Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, "preferred" residues associated with high affinity binding to an allele-specific HLA molecule or to multiple HLA molecules within a superfamily are inserted.

To ensure that an analog peptide, when used as a vaccine, actually elicits a CTL response to the native epitope *in vivo* (or, in the case of class II epitopes, elicits helper T cells that cross-react with the wild type peptides), the analog peptide may be used to immunize T cells *in vitro* from individuals of the appropriate HLA allele. Thereafter, the immunized cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. It will be desirable to use as antigen presenting cells, cells that have been either infected, or transfected with the appropriate genes, or, in the case of class II epitopes, cells that have been pulsed with whole protein antigens, to establish whether endogenously produced antigen is also recognized by the relevant T cells.

Another embodiment of the invention is to create analogs of weak binding peptides, to thereby ensure adequate numbers of cross-reactive cellular binders. Class I binding peptides exhibiting binding affinities of 500-5000 nM, and carrying an acceptable but suboptimal primary anchor residue at one or both positions can be "fixed" by substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

Another embodiment for generating effective peptide analogs involves the substitution of residues that have an adverse impact on peptide stability or solubility in, *e.g.*, a liquid environment. This substitution may occur at any position of the peptide epitope. For example, a cysteine can be substituted out in favor of α-amino butyric acid ("B" in the single letter abbreviations for peptide sequences listed herein). Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting α-amino butyric acid for cysteine not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances (*see, e.g.*, the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999).

IV.G. Computer Screening of Protein Sequences from Disease-Related Antigens for Supermotif- or Motif-Bearing Peptides

In order to identify supermotif- or motif-bearing epitopes in a target antigen, a native protein sequence, *e.g.*, a tumor-associated antigen, or sequences from an infectious organism, or a donor tissue for transplantation, is screened using a means for computing, such as an intellectual calculation or a

computer, to determine the presence of a supermotif or motif within the sequence. The information obtained from the analysis of native peptide can be used directly to evaluate the status of the native peptide or may be utilized subsequently to generate the peptide epitope.

Computer programs that allow the rapid screening of protein sequences for the occurrence of the subject supermotifs or motifs are encompassed by the present invention; as are programs that permit the generation of analog peptides. These programs are implemented to analyze any identified amino acid sequence or operate on an unknown sequence and simultaneously determine the sequence and identify motif-bearing epitopes thereof; analogs can be simultaneously determined as well. Generally, the identified sequences will be from a pathogenic organism or a tumor-associated peptide. In the present invention, the target TAA molecules include, without limitation, PSA, PSM, PAP, and hK2.

It is important that the selection criteria utilized for prediction of peptide binding are as accurate as possible, to correlate most efficiently with actual binding. Prediction of peptides that bind, for example, to HLA-A*0201, on the basis of the presence of the appropriate primary anchors, is positive at about a 30% rate (*see, e.g.*, Ruppert, J. *et al. Cell* 74:929, 1993). However, by extensively analyzing peptide-HLA binding data disclosed herein, data in related patent applications, and data in the art, the present inventors have developed a number of allele-specific polynomial algorithms that dramatically increase the predictive value over identification on the basis of the presence of primary anchor residues alone. These algorithms take into account not only the presence or absence of primary anchors, but also consider the positive or deleterious presence of secondary anchor residues (to account for the impact of different amino acids at different positions). The algorithms are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA interactions can be approximated as a linear polynomial function of the type:

$$\Delta G = a_{1i} \times a_{2i} \times a_{3i} \ldots \times a_{ni}$$

where a_{ji} is a coefficient that represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. An important assumption of this method is that the effects at each position are essentially independent of each other. This assumption is justified by studies that demonstrated that peptides are bound to HLA molecules and recognized by T cells in essentially an extended conformation. Derivation of specific algorithm coefficients has been described, for example, in Gulukota, K. *et al., J. Mol. Biol.* 267:1258, 1997.

Additional methods to identify preferred peptide sequences, which also make use of specific motifs, include the use of neural networks and molecular modeling programs (*see, e.g.*, Milik *et al., Nature Biotechnology* 16:753, 1998; Altuvia *et al., Hum. Immunol.* 58:1, 1997; Altuvia *et al, J. Mol. Biol.* 249:244, 1995; Buus, S. *Curr. Opin. Immunol.* 11:209-213, 1999; Brusic, V. *et al., Bioinformatics* 14:121-130, 1998; Parker *et al., J. Immunol.* 152:163, 1993; Meister *et al., Vaccine* 13:581, 1995; Hammer *et al., J. Exp. Med.* 180:2353, 1994; Sturniolo *et al., Nature Biotechnol.* 17:555 1999).

For example, it has been shown that in sets of A*0201 motif-bearing peptides containing at least one preferred secondary anchor residue while avoiding the presence of any deleterious secondary anchor residues, 69% of the peptides will bind A*0201 with an IC_{50} less than 500 nM (Ruppert, J. *et al. Cell* 74:929, 1993). These algorithms are also flexible in that cut-off scores may be adjusted to select sets of peptides with greater or lower predicted binding properties, as desired.

In utilizing computer screening to identify peptide epitopes, a protein sequence or translated sequence may be analyzed using software developed to search for motifs, for example the 'FINDPATTERNS' program (Devereux, *et al. Nucl. Acids Res.* 12:387-395, 1984) or MotifSearch 1.4 software program (D. Brown, San Diego, CA) to identify potential peptide sequences containing appropriate HLA binding motifs. The identified peptides can be scored using customized polynomial algorithms to predict their capacity to bind specific HLA class I or class II alleles. As appreciated by one of ordinary skill in the art, a large array of computer programming software and hardware options are available in the relevant art which can be employed to implement the motifs of the invention in order to evaluate (*e.g.*, without limitation, to identify epitopes, identify epitope concentration per peptide length, or to generate analogs) known or unknown peptide sequences.

In accordance with the procedures described above, prostate cancer-associated antigen peptide epitopes and analogs thereof that are able to bind HLA supertype groups or allele-specific HLA molecules are identified.

IV.H. Preparation of Peptide Epitopes

Peptides in accordance with the invention can be prepared synthetically, by recombinant DNA technology or chemical synthesis, or from natural sources such as native tumors or pathogenic organisms. Peptide epitopes may be synthesized individually or as polyepitopic peptides. Although the peptide will preferably be substantially free of other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptides may be synthetically conjugated to native fragments or particles.

The peptides in accordance with the invention can be a variety of lengths, and either in their neutral (uncharged) forms or in forms which are salts. The peptides in accordance with the invention are either free of modifications such as glycosylation, side chain oxidation, or phosphorylation; or they contain these modifications, subject to the condition that modifications do not destroy the biological activity of the peptides as described herein.

When possible, it may be desirable to optimize HLA class I binding epitopes of the invention, such as can be used in a polyepitopic construct, to a length of about 8 to about 13 amino acid residues, often 8 to 11, preferably 9 to 10. HLA class II binding peptide epitopes of the invention may be optimized to a length of about 6 to about 30 amino acids in length, preferably to between about 13 and about 20 residues. Preferably, the peptide epitopes are commensurate in size with endogenously processed pathogen-derived peptides or tumor cell peptides that are bound to the relevant HLA molecules, however, the identification and preparation of peptides that comprise epitopes of the invention can also be carried out using the techniques described herein.

In alternative embodiments, epitopes of the invention can be linked as a polyepitopic peptide, or as a minigene that encodes a polyepitopic peptide.

In another embodiment, it is preferred to identify native peptide regions that contain a high concentration of class I and/or class II epitopes. Such a sequence is generally selected on the basis that it contains the greatest number of epitopes per amino acid length. It is to be appreciated that epitopes can be present in a nested or overlapping manner, *e.g.* a 10 amino acid long peptide could contain two 9 amino acid long epitopes and one 10 amino acid long epitope; upon intracellular processing, each epitope can be

exposed and bound by an HLA molecule upon administration of such a peptide. This larger, preferably multi-epitopic, peptide can be generated synthetically, recombinantly, or via cleavage from the native source.

The peptides of the invention can be prepared in a wide variety of ways. For the preferred relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. (*See*, for example, Stewart & Young, SOLID PHASE PEPTIDE SYNTHESIS, 2D. ED., Pierce Chemical Co., 1984). Further, individual peptide epitopes can be joined using chemical ligation to produce larger peptides that are still within the bounds of the invention.

Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989). Thus, recombinant polypeptides which comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

The nucleotide coding sequence for peptide epitopes of the preferred lengths contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, *et al., J. Am. Chem. Soc.* 103:3185 (1981). Peptide analogs can be made simply by substituting the appropriate and desired nucleic acid base(s) for those that encode the native peptide sequence; exemplary nucleic acid substitutions are those that encode an amino acid defined by the motifs/supermotifs herein. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Of course, yeast, insect or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

IV.I. Assays to Detect T-Cell Responses

Once HLA binding peptides are identified, they can be tested for the ability to elicit a T-cell response. The preparation and evaluation of motif-bearing peptides are described in PCT publications WO 94/20127 and WO 94/03205. Briefly, peptides comprising epitopes from a particular antigen are synthesized and tested for their ability to bind to the appropriate HLA proteins. These assays may involve evaluating the binding of a peptide of the invention to purified HLA class I molecules in relation to the binding of a radioiodinated reference peptide. Alternatively, cells expressing empty class I molecules (*i.e.* lacking peptide therein) may be evaluated for peptide binding by immunofluorescent staining and flow microfluorimetry. Other assays that may be used to evaluate peptide binding include peptide-dependent

class I assembly assays and/or the inhibition of CTL recognition by peptide competition. Those peptides that bind to the class I molecule, typically with an affinity of 500 nM or less, are further evaluated for their ability to serve as targets for CTLs derived from infected or immunized individuals, as well as for their capacity to induce primary *in vitro* or *in vivo* CTL responses that can give rise to CTL populations capable of reacting with selected target cells associated with a disease.

Analogous assays are used for evaluation of HLA class II binding peptides. HLA class II motif-bearing peptides that are shown to bind, typically at an affinity of 1000 nM or less, are further evaluated for the ability to stimulate HTL responses.

Conventional assays utilized to detect T cell responses include proliferation assays, lymphokine secretion assays, direct cytotoxicity assays, and limiting dilution assays. For example, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations. Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells. Alternatively, mutant non-human mammalian cell lines that are deficient in their ability to load class I molecules with internally processed peptides and that have been transfected with the appropriate human class I gene, may be used to test for the capacity of the peptide to induce *in vitro* primary CTL responses.

Peripheral blood mononuclear cells (PBMCs) may be used as the responder cell source of CTL precursors. The appropriate antigen-presenting cells are incubated with peptide, after which the peptide-loaded antigen-presenting cells are then incubated with the responder cell population under optimized culture conditions. Positive CTL activation can be determined by assaying the culture for the presence of CTLs that kill radio-labeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed forms of the antigen from which the peptide sequence was derived.

Additionally, a method has been devised which allows direct quantification of antigen-specific T cells by staining with Fluorescein-labelled HLA tetrameric complexes (Altman, J. D. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:10330, 1993; Altman, J. D. *et al.*, *Science* 274:94, 1996). Other relatively recent technical developments include staining for intracellular lymphokines, and interferon-γ release assays or ELISPOT assays. Tetramer staining, intracellular lymphokine staining and ELISPOT assays all appear to be at least 10-fold more sensitive than more conventional assays (Lalvani, A. *et al.*, *J. Exp. Med.* 186:859, 1997; Dunbar, P. R. *et al.*, *Curr. Biol.* 8:413, 1998; Murali-Krishna, K. *et al.*, *Immunity* 8:177, 1998).

HTL activation may also be assessed using such techniques known to those in the art such as T cell proliferation and secretion of lymphokines, *e.g.* IL-2 (*see, e.g.* Alexander *et al.*, Immunity 1:751-761, 1994).

Alternatively, immunization of HLA transgenic mice can be used to determine immunogenicity of peptide epitopes. Several transgenic mouse models including mice with human A2.1, A11 (which can additionally be used to analyze HLA-A3 epitopes), and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed. Additional transgenic mouse models with other HLA alleles may be generated as necessary. The mice may be immunized with peptides emulsified in Incomplete Freund's Adjuvant and the resulting T cells tested for their capacity to recognize peptide-pulsed target cells and

target cells transfected with appropriate genes. CTL responses may be analyzed using cytotoxicity assays described above. Similarly, HTL responses may be analyzed using such assays as T cell proliferation or secretion of lymphokines.

IV.J. Use of Peptide Epitopes as Diagnostic Agents and for Evaluating Immune Responses

In one embodiment of the invention, HLA class I and class II binding peptides as described herein are used as reagents to evaluate an immune response. The immune response to be evaluated is induced by using as an immunogen any agent that may result in the production of antigen-specific CTLs or HTLs that recognize and bind to the peptide epitope(s) to be employed as the reagent. The peptide reagent need not be used as the immunogen. Assay systems that are used for such an analysis include relatively recent technical developments such as tetramers, staining for intracellular lymphokines and interferon release assays, or ELISPOT assays.

For example, peptides of the invention are used in tetramer staining assays to assess peripheral blood mononuclear cells for the presence of antigen-specific CTLs following exposure to a tumor cell antigen or an immunogen. The HLA-tetrameric complex is used to directly visualize antigen-specific CTLs (*see, e.g.*, Ogg *et al.*, *Science* 279:2103-2106, 1998; and Altman *et al.*, *Science* 174:94-96, 1996) and determine the frequency of the antigen-specific CTL population in a sample of peripheral blood mononuclear cells. A tetramer reagent using a peptide of the invention is generated as follows: A peptide that binds to an HLA molecule is refolded in the presence of the corresponding HLA heavy chain and β_2-microglobulin to generate a trimolecular complex. The complex is biotinylated at the carboxyl terminal end of the heavy chain at a site that was previously engineered into the protein. Tetramer formation is then induced by the addition of streptavidin. By means of fluorescently labeled streptavidin, the tetramer can be used to stain antigen-specific cells. The cells can then be identified, for example, by flow cytometry. Such an analysis may be used for diagnostic or prognostic purposes. Cells identified by the procedure can also be used for therapeutic purposes.

Peptides of the invention are also used as reagents to evaluate immune recall responses (*see, e.g.*, Bertoni *et al.*, *J. Clin. Invest.* 100:503-513, 1997 and Penna *et al.*, *J. Exp. Med.* 174:1565-1570, 1991). For example, patient PBMC samples from individuals with cancer are analyzed for the presence of antigen-specific CTLs or HTLs using specific peptides. A blood sample containing mononuclear cells can be evaluated by cultivating the PBMCs and stimulating the cells with a peptide of the invention. After an appropriate cultivation period, the expanded cell population can be analyzed, for example, for CTL or for HTL activity.

The peptides are also used as reagents to evaluate the efficacy of a vaccine. PBMCs obtained from a patient vaccinated with an immunogen are analyzed using, for example, either of the methods described above. The patient is HLA typed, and peptide epitope reagents that recognize the allele-specific molecules present in that patient are selected for the analysis. The immunogenicity of the vaccine is indicated by the presence of epitope-specific CTLs and/or HTLs in the PBMC sample.

The peptides of the invention are also used to make antibodies, using techniques well known in the art (see, *e.g.* *CURRENT PROTOCOLS IN IMMUNOLOGY*, Wiley/Greene, NY; and *Antibodies A Laboratory Manual*, Harlow and Lane, Cold Spring Harbor Laboratory Press, 1989), which may be useful

as reagents to diagnose or monitor cancer. Such antibodies include those that recognize a peptide in the context of an HLA molecule, *i.e.*, antibodies that bind to a peptide-MHC complex.

IV.K. Vaccine Compositions

Vaccines and methods of preparing vaccines that contain an immunogenically effective amount of one or more peptides as described herein are further embodiments of the invention. Once appropriately immunogenic epitopes have been defined, they can be sorted and delivered by various means, herein referred to as "vaccine" compositions. Such vaccine compositions can include, for example, lipopeptides (*e.g.*,Vitiello, A. *et al.*, *J. Clin. Invest.* 95:341, 1995), peptide compositions encapsulated in poly(DL-lactide-co-glycolide) ("PLG") microspheres (*see, e.g.*, Eldridge, *et al.*, *Molec. Immunol.* 28:287-294, 1991: Alonso *et al.*, *Vaccine* 12:299-306, 1994; Jones *et al.*, *Vaccine* 13:675-681, 1995), peptide compositions contained in immune stimulating complexes (ISCOMS) (*see, e.g.*, Takahashi *et al.*, *Nature* 344:873-875, 1990; Hu *et al.*, *Clin Exp Immunol.* 113:235-243, 1998), multiple antigen peptide systems (MAPs) (*see e.g.*, Tam, J. P., *Proc. Natl. Acad. Sci. U.S.A.* 85:5409-5413, 1988; Tam, J.P., *J. Immunol. Methods* 196:17-32, 1996), peptides formulated as multivalent peptides; peptides for use in ballistic delivery systems, typically crystallized peptides, viral delivery vectors (Perkus, M. E. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 379, 1996; Chakrabarti, S. *et al.*, *Nature* 320:535, 1986; Hu, S. L. *et al.*, *Nature* 320:537, 1986; Kieny, M.-P. *et al.*, *AIDS Bio/Technology* 4:790, 1986; Top, F. H. *et al.*, *J. Infect. Dis.* 124:148, 1971; Chanda, P. K. *et al.*, *Virology* 175:535, 1990), particles of viral or synthetic origin (*e.g.*, Kofler, N. *et al.*, *J. Immunol. Methods.* 192:25, 1996; Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993; Falo, L. D., Jr. *et al.*, *Nature Med.* 7:649, 1995), adjuvants (Warren, H. S., Vogel, F. R., and Chedid, L. A. *Annu. Rev. Immunol.* 4:369, 1986; Gupta, R. K. *et al.*, *Vaccine* 11:293, 1993), liposomes (Reddy, R. *et al.*, *J. Immunol.* 148:1585, 1992; Rock, K. L., *Immunol. Today* 17:131, 1996), or, naked or particle absorbed cDNA (Ulmer, J. B. *et al.*, *Science* 259:1745, 1993; Robinson, H. L., Hunt, L. A., and Webster, R. G., *Vaccine* 11:957, 1993; Shiver, J. W. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 423, 1996; Cease, K. B., and Berzofsky, J. A., *Annu. Rev. Immunol.* 12:923, 1994 and Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993). Toxin-targeted delivery technologies, also known as receptor mediated targeting, such as those of Avant Immunotherapeutics, Inc. (Needham, Massachusetts) may also be used.

Vaccines of the invention include nucleic acid-mediated modalities. DNA or RNA encoding one or more of the peptides of the invention can also be administered to a patient. This approach is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivicaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (*see, e.g.*, U.S. Patent No. 5,922,687).

For therapeutic or prophylactic immunization purposes, the peptides of the invention can also be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. As an example of this approach, vaccinia virus is used as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into a host

bearing a tumor, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL and/or HTL response. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.*, *Nature* 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, *e.g.* adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, will be apparent to those skilled in the art from the description herein.

Furthermore, vaccines in accordance with the invention encompass compositions of one or more of the claimed peptides. A peptide can be present in a vaccine individually. Alternatively, the peptide can exist as a homopolymer comprising multiple copies of the same peptide, or as a heteropolymer of various peptides. Polymers have the advantage of increased immunological reaction and, where different peptide epitopes are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the pathogenic organism or tumor-related peptide targeted for an immune response. The composition can be a naturally occurring region of an antigen or can be prepared, *e.g.*, recombinantly or by chemical synthesis.

Carriers that can be used with vaccines of the invention are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. The vaccines can contain a physiologically tolerable (*i.e.*, acceptable) diluent such as water, or saline, preferably phosphate buffered saline. The vaccines also typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are examples of materials well known in the art. Additionally, as disclosed herein, CTL responses can be primed by conjugating peptides of the invention to lipids, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P_3CSS).

Upon immunization with a peptide composition in accordance with the invention, via injection, aerosol, oral, transdermal, transmucosal, intrapleural, intrathecal, or other suitable routes, the immune system of the host responds to the vaccine by producing large amounts of CTLs and/or HTLs specific for the desired antigen. Consequently, the host becomes at least partially immune to later infection, or at least partially resistant to developing an ongoing chronic infection, or derives at least some therapeutic benefit when the antigen was tumor-associated.

In some embodiments, it may be desirable to combine the class I peptide components with components that induce or facilitate neutralizing antibody and or helper T cell responses to the target antigen of interest. A preferred embodiment of such a composition comprises class I and class II epitopes in accordance with the invention. An alternative embodiment of such a composition comprises a class I and/or class II epitope in accordance with the invention, along with a cross-binding HLA class II molecule such as PADRE™ (Epimmune, San Diego, CA) molecule (described, for example, in U.S. Patent Number 5,736,142).

A vaccine of the invention can also include antigen-presenting cells (APC), such as dendritic cells (DC), as a vehicle to present peptides of the invention. Vaccine compositions can be created *in vitro*, following dendritic cell mobilization and harvesting, whereby loading of dendritic cells occurs *in vitro*. For example, dendritic cells are transfected, *e.g.*, with a minigene in accordance with the invention,

or are pulsed with peptides. The dendritic cell can then be administered to a patient to elicit immune responses *in vivo*.

Vaccine compositions, either DNA- or peptide-based, can also be administered *in vivo* in combination with dendritic cell mobilization whereby loading of dendritic cells occurs *in vivo*.

Antigenic peptides are used to elicit a CTL and/or HTL response *ex vivo*, as well. The resulting CTL or HTL cells, can be used to treat tumors in patients that do not respond to other conventional forms of therapy, or will not respond to a therapeutic vaccine peptide or nucleic acid in accordance with the invention. *Ex vivo* CTL or HTL responses to a particular tumor-associated antigen are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells, such as dendritic cells, and the appropriate immunogenic peptide. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cell (an infected cell or a tumor cell). Transfected dendritic cells may also be used as antigen presenting cells.

The vaccine compositions of the invention can also be used in combination with other treatments used for cancer, including use in combination with immune adjuvants such as IL-2, IL-12, GM-CSF, and the like.

Preferably, the following principles are utilized when selecting an array of epitopes for inclusion in a polyepitopic composition for use in a vaccine, or for selecting discrete epitopes to be included in a vaccine and/or to be encoded by nucleic acids such as a minigene. It is preferred that each of the following principles are balanced in order to make the selection. The multiple epitopes to be incorporated in a given vaccine composition may be, but need not be, contiguous in sequence in the native antigen from which the epitopes are derived.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For HLA Class I this includes 3-4 epitopes that come from at least one TAA. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one TAA (*see e.g.*, Rosenberg *et al.*, *Science* 278:1447-1450). Epitopes from one TAA may be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs as described, *e.g.*, in Example 15.

2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, often 200 nM or less; and for Class II an IC_{50} of 1000 nM or less.

3.) Sufficient supermotif bearing-peptides, or a sufficient array of allele-specific motif-bearing peptides, are selected to give broad population coverage. For example, it is preferable to have at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess the breadth, or redundancy of, population coverage.

4.) When selecting epitopes from cancer-related antigens it is often useful to select analogs because the patient may have developed tolerance to the native epitope. When selecting epitopes for infectious disease-related antigens it is preferable to select either native or analoged epitopes.

5.) Of particular relevance are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A nested peptide sequence can comprise both HLA class I and HLA class II epitopes. When providing nested epitopes, a general objective is to provide the greatest number of epitopes per sequence. Thus, an aspect is to avoid providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a multi-epitopic sequence, such as a sequence comprising nested epitopes, it is generally important to screen the sequence in order to insure that it does not have pathological or other deleterious biological properties.

6.) If a polyepitopic protein is created, or when creating a minigene, an objective is to generate the smallest peptide that encompasses the epitopes of interest. This principle is similar, if not the same as that employed when selecting a peptide comprising nested epitopes. However, with an artificial polyepitopic peptide, the size minimization objective is balanced against the need to integrate any spacer sequences between epitopes in the polyepitopic protein. Spacer amino acid residues can, for example, be introduced to avoid junctional epitopes (an epitope recognized by the immune system, not present in the target antigen, and only created by the man-made juxtaposition of epitopes), or to facilitate cleavage between epitopes and thereby enhance epitope presentation. Junctional epitopes are generally to be avoided because the recipient may generate an immune response to that non-native epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

IV.K.1. Minigene Vaccines

A number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the peptides of the invention are a particularly useful embodiment of the invention. Epitopes for inclusion in a minigene are preferably selected according to the guidelines set forth in the previous section. A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding a peptide comprising one or multiple epitopes of the invention.

The use of multi-epitope minigenes is described below and in, *e.g.*, co-pending application U.S.S.N. 09/311,784; Ishioka *et al.*, *J. Immunol.* 162:3915-3925, 1999; An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997; Thomson, S. A. *et al.*, *J. Immunol.* 157:822, 1996; Whitton, J. L. *et al.*, *J. Virol.* 67:348, 1993; Hanke, R. *et al.*, *Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding supermotif- and/or motif-bearing PSA, PSM, PAP, and hK2 epitopes derived from multiple regions of one or more of the prostate cancer-associated antigens, the PADRE™ universal helper T cell epitope (or multiple HTL epitopes from PSA, PSM, PAP, and hK2), and an endoplasmic reticulum-translocating signal sequence can be engineered. A vaccine may also comprise epitopes that are derived from other TAAs.

The immunogenicity of a multi-epitopic minigene can be tested in transgenic mice to evaluate the magnitude of CTL induction responses against the epitopes tested. Further, the immunogenicity of DNA-encoded epitopes *in vivo* can be correlated with the *in vitro* responses of specific CTL lines against target cells transfected with the DNA plasmid. Thus, these experiments can show that

the minigene serves to both: 1.) generate a CTL response and 2.) that the induced CTLs recognized cells expressing the encoded epitopes.

For example, to create a DNA sequence encoding the selected epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes may be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that can be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes may be improved by including synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the scope of the invention.

The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus (hCMV) promoter. See, *e.g.*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can

be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (*e.g.*, IL-2, IL-12, GM-CSF), cytokine-inducing molecules (*e.g.*, LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins (*e.g.*, PADRE™, Epimmune, San Diego, CA). Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (*e.g.* TGF-β) may be beneficial in certain diseases.

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffered saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids, glycolipids, and fusogenic liposomes can also be used in the formulation (see, *e.g.*, as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et al., Proc. Nat'l Acad. Sci. USA* 84:7413 (1987). In addition, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

Target cell sensitization can be used as a functional assay for expression and HLA class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (^{51}Cr) labeled and used as target cells for epitope-specific CTL lines; cytolysis, detected by ^{51}Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes. Expression of HTL epitopes may be evaluated in an analogous manner using assays to assess HTL activity.

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (*e.g.*, IM for DNA in PBS, intraperitoneal (IP) for lipid-complexed DNA). Twenty-one days after immunization, splenocytes are

harvested and restimulated for one week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, ^{51}Cr-labeled target cells using standard techniques. Lysis of target cells that were sensitized by HLA loaded with peptide epitopes, corresponding to minigene-encoded epitopes, demonstrates DNA vaccine function for *in vivo* induction of CTLs. Immunogenicity of HTL epitopes is evaluated in transgenic mice in an analogous manner.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

Minigenes can also be delivered using other bacterial or viral delivery systems well known in the art, *e.g.*, an expression construct encoding epitopes of the invention can be incorporated into a viral vector such as vaccinia.

IV.K.2. Combinations of CTL Peptides with Helper Peptides

Vaccine compositions comprising the peptides of the present invention can be modified to provide desired attributes, such as improved serum half-life, or to enhance immunogenicity.

For instance, the ability of a peptide to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. The use of T helper epitopes in conjunction with CTL epitopes to enhance immunogenicity is illustrated, for example, in the co-pending applications U.S.S.N. 08/820,360, U.S.S.N. 08/197,484, and U.S.S.N. 08/464,234.

Although a CTL peptide can be directly linked to a T helper peptide, often CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, *e.g.*, Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues and sometimes 10 or more residues. The CTL peptide epitope can be linked to the T helper peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide may be acylated.

In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in the majority of the population. This can be accomplished by selecting amino acid sequences that bind to many, most, or all of the HLA class II molecules. These are known as "loosely HLA-restricted" or "promiscuous" T helper sequences. Examples of peptides that are promiscuous include sequences from antigens such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE), *Plasmodium falciparum* circumsporozoite (CS) protein at positions 378-398 (DIEKKIAKMEKASSVFNVVNS), and *Streptococcus* 18kD protein at positions 116 (GAVDSILGGVATYGAA). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs.

Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (*see, e.g.*, PCT publication WO 95/07707). These synthetic compounds called Pan-DR-binding epitopes (*e.g.*, PADRE™, Epimmune, Inc., San Diego, CA) are designed to most preferrably bind most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVAAWTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and "a" is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals; regardless of their HLA type. An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

HTL peptide epitopes can also be modified to alter their biological properties. For example, they can be modified to include D-amino acids to increase their resistance to proteases and thus extend their serum half life, or they can be conjugated to other molecules such as lipids, proteins, carbohydrates, and the like to increase their biological activity. For example, a T helper peptide can be conjugated to one or more palmitic acid chains at either the amino or carboxyl termini.

IV.K.3. Combinations of CTL Peptides with T Cell Priming Agents

In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which primes cytotoxic T lymphocytes. Lipids have been identified as agents capable of priming CTL *in vivo* against viral antigens. For example, palmitic acid residues can be attached to the ε-and α- amino groups of a lysine residue and then linked, *e.g.*, via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant, *e.g.*, incomplete Freund's adjuvant. A preferred immunogenic composition comprises palmitic acid attached to ε- and α- amino groups of Lys, which is attached via linkage, *e.g.*, Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P_3CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide (*see, e.g.*, Deres, *et al., Nature* 342:561, 1989). Peptides of the invention can be coupled to P_3CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P_3CSS-conjugated epitopes, two such compositions can be combined to more effectively elicit both humoral and cell-mediated responses.

CTL and/or HTL peptides can also be modified by the addition of amino acids to the termini of a peptide to provide for ease of linking peptides one to another, for coupling to a carrier support or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide, particularly class I peptides. However, it is to be noted that modification at the carboxyl terminus of a CTL epitope may, in some cases, alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural

sequence by being modified by terminal-NH_2 acylation, *e.g.*, by alkanoyl (C_1-C_{20}) or thioglycolyl acetylation, terminal-carboxyl amidation, *e.g.*, ammonia, methylamine, *etc.* In some instances these modifications may provide sites for linking to a support or other molecule.

IV.K.4. Vaccine Compositions Comprising DC Pulsed with CTL and/or HTL Peptides

An embodiment of a vaccine composition in accordance with the invention comprises *ex vivo* administration of a cocktail of epitope-bearing peptides to PBMC, or isolated DC therefrom, from the patient's blood. A pharmaceutical to facilitate harvesting of DC can be used, such as Progenipoietin™ (Monsanto, St. Louis, MO) or GM-CSF/IL-4. After pulsing the DC with peptides and prior to reinfusion into patients, the DC are washed to remove unbound peptides. In this embodiment, a vaccine comprises peptide-pulsed DCs which present the pulsed peptide epitopes complexed with HLA molecules on their surfaces.

The DC can be pulsed *ex vivo* with a cocktail of peptides, some of which stimulate CTL response to one or more antigens of interest, *e.g.*, prostate-associated antigens such as PSA, PSM, PAP, kallikrein, and the like. Optionally, a helper T cell peptide such as a PADRE™ family molecule, can be included to facilitate the CTL response.

IV.L. Administration of Vaccines for Therapeutic or Prophylactic Purposes

The peptides of the present invention and pharmaceutical and vaccine compositions of the invention are typically used therapeutically to treat cancer, particularly prostate cancer. Vaccine compositions containing the peptides of the invention are typically administered to a prostate cancer patient who has a malignancy associated with expression of one or more prostate-associated antigens. Alternatively, vaccine compositions can be administered to an individual susceptible to, or otherwise at risk for developing prostate cancer.

In therapeutic applications, peptide and/or nucleic acid compositions are administered to a patient in an amount sufficient to elicit an effective CTL and/or HTL response to the tumor antigen and to cure or at least partially arrest or slow symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.*, the particular composition administered, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

As noted above, peptides comprising CTL and/or HTL epitopes of the invention induce immune responses when presented by HLA molecules and contacted with a CTL or HTL specific for an epitope comprised by the peptide. The peptides (or DNA encoding them) can be administered individually or as fusions of one or more peptide sequences. The manner in which the peptide is contacted with the CTL or HTL is not critical to the invention. For instance, the peptide can be contacted with the CTL or HTL either *in vivo* or *in vitro*. If the contacting occurs *in vivo*, the peptide itself can be administered to the patient, or other vehicles, *e.g.*, DNA vectors encoding one or more peptides, viral vectors encoding the peptide(s), liposomes and the like, can be used, as described herein.

When the peptide is contacted *in vitro*, the vaccinating agent can comprise a population of cells, *e.g.*, peptide-pulsed dendritic cells, or TAA-specific CTLs, which have been induced by pulsing antigen-presenting cells *in vitro* with the peptide or by transfecting antigen-presenting cells with a minigene of the invention. Such a cell population is subsequently administered to a patient in a therapeutically effective dose.

For therapeutic use, administration should generally begin at the first diagnosis of cancer. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. The embodiment of the vaccine composition (*i.e.*, including, but not limited to embodiments such as peptide cocktails, polyepitopic polypeptides, minigenes, or TAA-specific CTLs or pulsed dendritic cells) delivered to the patient may vary according to the stage of the disease or the patient's health status. For example, a vaccine comprising TAA-specific CTLs may be more efficacious in killing tumor cells in patients with advanced disease than alternative embodiments.

The vaccine compositions of the invention may also be used therapeutically in combination with treatments such as surgery. An example is a situation in which a patient has undergone surgery to remove a primary tumor and the vaccine is then used to slow or prevent recurrence and/or metastasis.

Where susceptible individuals, *e.g.*, individuals who may be diagnosed as being genetically pre-disposed to developing a prostate tumor, are identified prior to diagnosis of cancer, the composition can be targeted to them, thus minimizing the need for administration to a larger population.

The dosage for an initial therapeutic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1,000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg. Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. Initial doses followed by boosting doses at established intervals, *e.g.*, from four weeks to six months, may be required, possibly for a prolonged period of time to effectively treat a patient. Boosting dosages of between about 1.0 μg to about 50,000 μg of peptide pursuant to a boosting regimen over weeks to months may be administered depending upon the patient's response and condition as determined by measuring the specific activity of CTL and HTL obtained from the patient's blood.

Administration should continue until at least clinical symptoms or laboratory tests indicate that the tumor has been eliminated or that the tumor cell burden has been substantially reduced and for a period thereafter. The dosages, routes of administration, and dose schedules are adjusted in accordance with methodologies known in the art.

In certain embodiments, peptides and compositions of the present invention are employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, as a result of the minimal amounts of extraneous substances and the relative nontoxic nature of the peptides in preferred compositions of the invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions relative to these stated dosage amounts.

The vaccine compositions of the invention can also be used as prophylactic agents. For example, the compositions can be administered to individuals at risk of developing prostate cancer. Generally the dosage for an initial prophylactic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or

50,000 µg. Dosage values for a human typically range from about 500 µg to about 50,000 µg per 70 kilogram patient. This is followed by boosting dosages of between about 1.0 µg to about 50,000 µg of peptide administered at defined intervals from about four weeks to six months after the initial administration of vaccine. The immunogenicity of the vaccine may be assessed by measuring the specific activity of CTL and HTL obtained from a sample of the patient's blood.

The pharmaceutical compositions for therapeutic treatment are intended for parenteral, topical, oral, intrathecal, or local administration. Preferably, the pharmaceutical compositions are administered parentally, *e.g.*, intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*

The concentration of peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, *etc.*, in accordance with the particular mode of administration selected.

A human unit dose form of the peptide composition is typically included in a pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (*see, e.g.*, Remington's Pharmaceutical Sciences, 17th Edition, A. Gennaro, Editor, Mack Publishing Co., Easton, Pennsylvania, 1985).

The peptides of the invention may also be administered via liposomes, which serve to target the peptides to a particular tissue, such as lymphoid tissue, or to target selectively to infected cells, as well as to increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations, the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes either filled or decorated with a desired peptide of the invention can be directed to the site of lymphoid cells, where the liposomes then deliver the peptide compositions. Liposomes for use in accordance with the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size, acid lability and stability of the liposomes in the blood stream. A

variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka, *et al., Ann. Rev. Biophys. Bioeng.* 9:467 (1980), and U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

For targeting cells of the immune system, a ligand to be incorporated into the liposome can include, *e.g.*, antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, *etc.* in a dose which varies according to, *inter alia*, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, *e.g.*, lecithin for intranasal delivery.

IV.M. HLA EXPRESSION: IMPLICATIONS FOR T CELL-BASED IMMUNOTHERAPY

Disease progression in cancer and infectious disease

It is well recognized that a dynamic interaction between exists between host and disease, both in the cancer and infectious disease settings. In the infectious disease setting, it is well established that pathogens evolve during disease. The strains that predominate early in HIV infection are different from the ones that are associated with AIDS and later disease stages (NS versus S strains). It has long been hypothesized that pathogen forms that are effective in establishing infection may differ from the ones most effective in terms of replication and chronicity.

Similarly, it is widely recognized that the pathological process by which an individual succumbs to a neoplastic disease is complex. During the course of disease, many changes occur in cancer cells. The tumor accumulates alterations which are in part related to dysfunctional regulation of growth and differentiation, but also related to maximizing its growth potential, escape from drug treatment and/or the body's immunosurveillance. Neoplastic disease results in the accumulation of several different biochemical alterations of cancer cells, as a function of disease progression. It also results in significant levels of intra- and inter- cancer heterogeneity, particularly in the late, metastatic stage.

Familiar examples of cellular alterations affecting treatment outcomes include the outgrowth of radiation or chemotherapy resistant tumors during the course of therapy. These examples

parallel the emergence of drug resistant viral strains as a result of aggressive chemotherapy, *e.g.*, of chronic HBV and HIV infection, and the current resurgence of drug resistant organisms that cause Tuberculosis and Malaria. It appears that significant heterogeneity of responses is also associated with other approaches to cancer therapy, including anti-angiogenesis drugs, passive antibody immunotherapy, and active T cell-based immunotherapy. Thus, in view of such phenomena, epitopes from multiple disease-related antigens can be used in vaccines and therapeutics thereby counteracting the ability of diseased cells to mutate and escape treatment.

The interplay between disease and the immune system

One of the main factors contributing to the dynamic interplay between host and disease is the immune response mounted against the pathogen, infected cell, or malignant cell. In many conditions such immune responses control the disease. Several animal model systems and prospective studies of natural infection in humans suggest that immune responses against a pathogen can control the pathogen, prevent progression to severe disease and/or eliminate the pathogen. A common theme is the requirement for a multispecific T cell response, and that narrowly focused responses appear to be less effective. These observations guide skilled artisan as to embodiments of methods and compositions of the present invention that provide for a broad immune response.

In the cancer setting there are several findings that indicate that immune responses can impact neoplastic growth:

First, the demonstration in many different animal models, that anti-tumor T cells, restricted by MHC class I, can prevent or treat tumors.

Second, encouraging results have come from immunotherapy trials.

Third, observations made in the course of natural disease correlated the type and composition of T cell infiltrate within tumors with positive clinical outcomes (Coulie PG, *et al.* Antitumor immunity at work in a melanoma patient In *Advances in Cancer Research*, 213-242, 1999).

Finally, tumors commonly have the ability to mutate, thereby changing their immunological recognition. For example, the presence of monospecific CTL was also correlated with control of tumor growth, until antigen loss emerged (Riker A, *et al.*, Immune selection after antigen-specific immunotherapy of melanoma *Surgery*, Aug: 126(2):112-20, 1999; Marchand M, *et al.*, Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1 *Int. J. Cancer* 80(2):219-30, Jan. 18, 1999). Similarly, loss of beta 2 microglobulin was detected in 5/13 lines established from melanoma patients after receiving immunotherapy at the NCI (Restifo NP, *et al.*, Loss of functional Beta2 - microglobulin in metastatic melanomas from five patients receiving immunotherapy *Journal of the National Cancer Institute*, Vol. 88 (2), 100-108, Jan. 1996). It has long been recognized that HLA class I is frequently altered in various tumor types. This has led to a hypothesis that this phenomenon might reflect immune pressure exerted on the tumor by means of class I restricted CTL. The extent and degree of alteration in HLA class I expression appears to be reflective of past immune pressures, and may also have prognostic value (van Duinen SG, *et al.*, Level of HLA antigens in locoregional metastases and clinical course of the disease in patients with melanoma *Cancer Research* 48, 1019-1025, Feb. 1988; Möller P, *et al.*, Influence of major

histocompatibility complex class I and II antigens on survival in colorectal carcinoma *Cancer Research* 51, 729-736, Jan. 1991). Taken together, these observations provide a rationale for immmnotherapy of cancer and infectious disease, and suggest that effective strategies need to account for the complex series of pathological changes associated with disease.

The three main types of alterations in HLA expression in tumors and their functional significance

The level and pattern of expression of HLA class I antigens in tumors has been studied in many different tumor types and alterations have been reported in all types of tumors studied. The molecular mechanisms underlining HLA class I alterations have been demonstrated to be quite heterogeneous. They include alterations in the TAP/processing pathways, mutations of β2-microglobulin and specific HLA heavy chains, alterations in the regulatory elements controlling over class I expression and loss of entire chromosome sections. There are several reviews on this topic, *see, e.g.*, : Garrido F, *et al.*, Natural history of HLA expression during tumour development *Immunol Today* 14(10):491-499, 1993; Kaklamanis L, *et al.*, Loss of HLA class-I alleles, heavy chains and β2-microglobulin in colorectal cancer *Int. J. Cancer*, 51(3):379-85, May 28,1992. There are three main types of HLA Class I alteration (complete loss, allele-specific loss and decreased expression). The functional significance of each alteration is discussed separately:

Complete loss of HLA expression

Complete loss of HLA expression can result from a variety of different molecular mechanisms, reviewed in (Algarra I, *et al.*, The HLA crossroad in tumor immunology *Human Immunology* 61, 65-73, 2000; Browning M, *et al.*, Mechanisms of loss of HLA class I expression on colorectal tumor cells *Tissue Antigens* 47:364-371, 1996; Ferrone S, et al., Loss of HLA class I antigens by melanoma cells: molecular mechanisms, functional significance and clinical relevance *Immunology Today*, 16(10): 487-494, 1995; Garrido F, *et al.*, Natural history of HLA expression during tumour development *Immunology Today* 14(10):491-499, 1993; Tait, BD, HLA Class I expression on human cancer cells: Implications for effective immunotherapy *Hum Immunol* 61, 158-165, 2000). In functional terms, this type of alteration has several important implications.

While the complete absence of class I expression will eliminate CTL recognition of those tumor cells, the loss of HLA class I will also render the tumor cells extraordinary sensitive to lysis from NK cells (Ohnmacht, GA, *et al.*, Heterogeneity in expression of human leukocyte antigens and melanoma-associated antigens in advanced melanoma *J Cellular Phys* 182:332-338, 2000; Liunggren HG, *et al.*, Host resistance directed selectively against H-2 deficient lymphoma variants: Analysis of the mechanism *J. Exp. Med.*, Dec 1;162(6):1745-59, 1985; Maio M, *et al.*, Reduction in susceptibility to natural killer cell-mediated lysis of human FO-1 melanoma cells after induction of HLA class I antigen expression by transfection with B2m gene *J. Clin. Invest.* 88(1):282-9, July 1991; Schrier PI, *et al.*, Relationship between myc oncogene activation and MHC class I expression *Adv. Cancer Res.*, 60:181-246, 1993).

The complementary interplay between loss of HLA expression and gain in NK sensitivity is exemplified by the classic studies of Coulie and coworkers (Coulie, PG, *et al.*, Antitumor immunity at work in a melanoma patient. In *Advances in Cancer Research*, 213-242, 1999) which described the

evolution of a patient's immune response over the course of several years. Because of increased sensitivity to NK lysis, it is predicted that approaches leading to stimulation of innate immunity in general and NK activity in particular would be of special significance. An example of such approach is the induction of large amounts of dendritic cells (DC) by various hematopoietic growth factors, such as Flt3 ligand or ProGP. The rationale for this approach resides in the well known fact that dendritic cells produce large amounts of IL-12, one of the most potent stimulators for innate immunity and NK activity in particular. Alternatively, IL-12 is administered directly, or as nucleic acids that encode it. In this light, it is interesting to note that Flt3 ligand treatment results in transient tumor regression of a class I negative prostate murine cancer model (Ciavarra RP, *et al.*, Flt3-Ligand induces transient tumor regression in an ectopic treatment model of major histocompatibility complex-negative prostate cancer *Cancer Res* 60:2081-84, 2000). In this context, specific anti-tumor vaccines in accordance with the invention synergize with these types of hematopoietic growth factors to facilitate both CTL and NK cell responses, thereby appreciably impairing a cell's ability to mutate and thereby escape efficacious treatment. Thus, an embodiment of the present invention comprises a composition of the invention together with a method or composition that augments functional activity or numbers of NK cells. Such an embodiment can comprise a protocol that provides a composition of the invention sequentially with an NK-inducing modality, or contemporaneous with an NK-inducing modality.

Secondly, complete loss of HLA frequently occurs only in a fraction of the tumor cells, while the remainder of tumor cells continue to exhibit normal expression. In functional terms, the tumor would still be subject, in part, to direct attack from a CTL response; the portion of cells lacking HLA subject to an NK response. Even if only a CTL response were used, destruction of the HLA expressing fraction of the tumor has dramatic effects on survival times and quality of life.

It should also be noted that in the case of heterogeneous HLA expression, both normal HLA-expressing as well as defective cells are predicted to be susceptible to immune destruction based on "bystander effects." Such effects were demonstrated, e.g., in the studies of Rosendahl and colleagues that investigated in vivo mechanisms of action of antibody targeted superantigens (Rosendahl A, *et al.*, Perforin and IFN-gamma are involved in the antitumor effects of antibody-targeted superantigens *J. Immunol.* 160(11):5309-13, June 1, 1998). The bystander effect is understood to be mediated by cytokines elicited from, *e.g.*, CTLs acting on an HLA-bearing target cell, whereby the cytokines are in the environment of other diseased cells that are concomitantly killed.

Allele-specific loss

One of the most common types of alterations in class I molecules is the selective loss of certain alleles in individuals heterozygous for HLA. Allele-specific alterations might reflect the tumor adaptation to immune pressure, exerted by an immunodominant response restricted by a single HLA restriction element. This type of alteration allows the tumor to retain class I expression and thus escape NK cell recognition, yet still be susceptible to a CTL-based vaccine in accordance with the invention which comprises epitopes corresponding to the remaining HLA type. Thus, a practical solution to overcome the potential hurdle of allele-specific loss relies on the induction of multispecific responses. Just as the inclusion of multiple disease-associated antigens in a vaccine of the invention guards against mutations that

yield loss of a specific disease antigens, simultaneously targeting multiple HLA specificities and multiple disease-related antigens prevents disease escape by allele-specific losses.

Decrease in expression (allele-specific or not)

The sensitivity of effector CTL has long been demonstrated (Brower, RC, *et al.*, Minimal requirements for peptide mediated activation of CD8+ CTL *Mol. Immunol.*, 31;1285-93, 1994; Christnick, ET, *et al.* Low numbers of MHC class I-peptide complexes required to trigger a T cell response *Nature* 352:67-70, 1991; Sykulev, Y, *et al.*, Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response *Immunity*, 4(6):565-71, June 1996). Even a single peptide/MHC complex can result in tumor cells lysis and release of anti-tumor lymphokines. The biological significance of decreased HLA expression and possible tumor escape from immune recognition is not fully known. Nevertheless, it has been demonstrated that CTL recognition of as few as one MHC/peptide complex is sufficient to lead to tumor cell lysis.

Further, it is commonly observed that expression of HLA can be upregulated by gamma IFN, commonly secreted by effector CTL. Additionally, HLA class I expression can be induced in vivo by both alpha and beta IFN (Halloran, *et al.* Local T cell responses induce widespread MHC expression. *J Immunol* 148:3837, 1992; Pestka, S, *et al.*, Interferons and their actions *Annu. Rev. Biochem.* 56:727-77, 1987). Conversely, decreased levels of HLA class I expression also render cells more susceptible to NK lysis.

With regard to gamma IFN, Torres et al (Torres, MJ, *et al.*, Loss of an HLA haplotype in pancreas cancer tissue and its corresponding tumor derived cell line. *Tissue Antigens* 47:372-81, 1996) note that HLA expression is upregulated by gamma IFN in pancreatic cancer, unless a total loss of haplotype has occurred. Similarly, Rees and Mian note that allelic deletion and loss can be restored, at least partially, by cytokines such as IFN-gamma (Rees, R., *et al.* Selective MHC expression in tumours modulates adaptive and innate antitumour responses *Cancer Immunol Immunother* 48:374-81, 1999). It has also been noted that IFN-gamma treatment results in upregulation of class I molecules in the majority of the cases studied (Browning M, *et al.*, Mechanisms of loss of HLA class I expression on colorectal tumor cells. *Tissue Antigens* 47:364-71, 1996). Kaklamakis, et al. also suggested that adjuvant immunotherapy with IFN-gamma may be beneficial in the case of HLA class I negative tumors (Kaklamanis L, Loss of transporter in antigen processing 1 transport protein and major histocompatibility complex class I molecules in metastatic versus primary breast cancer. *Cancer Research* 55:5191-94, November 1995). It is important to underline that IFN-gamma production is induced and self-amplified by local inflammation/immunization (Halloran, *et al.* Local T cell responses induce widespread MHC expression *J. Immunol* 148:3837, 1992), resulting in large increases in MHC expressions even in sites distant from the inflammatory site.

Finally, studies have demonstrated that decreased HLA expression can render tumor cells more susceptible to NK lysis (Ohmmacht, GA, *et al.*, Heterogeneity in expression of human leukocyte antigens and melanoma-associated antigens in advanced melanoma *J Cellular Phys* 182:332-38, 2000; Liunggren HG, *et al.*, Host resistance directed selectively against H-2 deficient lymphoma variants: Analysis of the mechanism *J. Exp. Med.*, 162(6):1745-59, December 1, 1985; Maio M, *et al.*, Reduction in susceptibility to natural killer cell-mediated lysis of human FO-1 melanoma cells after induction of HLA

class I antigen expression by transfection with β2m gene *J. Clin. Invest.* 88(1):282-9, July 1991; Schrier PI, *et al.*, Relationship between myc oncogene activation and MHC class I expression *Adv. Cancer Res.*, 60:181-246, 1993). If decreases in HLA expression benefit a tumor because it facilitates CTL escape, but render the tumor susceptible to NK lysis, then a minimal level of HLA expression that allows for resistance to NK activity would be selected for (Garrido F, *et al.*, Implications for immunosurveillance of altered HLA class I phenotypes in human tumours *Immunol Today* 18(2):89-96, February 1997). Therefore, a therapeutic compositions or methods in accordance with the invention together with a treatment to upregulate HLA expression and/or treatment with high affinity T-cells renders the tumor sensitive to CTL destruction.

Frequency of alterations in HLA expression

The frequency of alterations in class I expression is the subject of numerous studies (Algarra I, *et al.*, The HLA crossroad in tumor immunology *Human Immunology* 61, 65-73, 2000). Rees and Mian estimate allelic loss to occur overall in 3-20% of tumors, and allelic deletion to occur in 15-50% of tumors. It should be noted that each cell carries two separate sets of class I genes, each gene carrying one HLA-A and one HLA-B locus. Thus, fully heterozygous individuals carry two different HLA-A molecules and two different HLA-B molecules. Accordingly, the actual frequency of losses for any specific allele could be as little as one quarter of the overall frequency. They also note that, in general, a gradient of expression exists between normal cells, primary tumors and tumor metastasis. In a study from Natali and coworkers (Natali PG, *et al.*, Selective changes in expression of HLA class I polymorphic determinants in human solid tumors *PNAS USA* 86:6719-6723, September 1989), solid tumors were investigated for total HLA expression, using W6/32 antibody, and for allele-specific expression of the A2 antigen, as evaluated by use of the BB7.2 antibody. Tumor samples were derived from primary cancers or metastasis, for 13 different tumor types, and scored as negative if less than 20%, reduced if in the 30-80% range, and normal above 80%. All tumors, both primary and metastatic, were HLA positive with W6/32. In terms of A2 expression, a reduction was noted in 16.1 % of the cases, and A2 was scored as undetectable in 39.4 % of the cases. Garrido and coworkers (Garrido F, *et al.*, Natural history of HLA expression during tumour development *Immunol Today* 14(10):491-99, 1993) emphasize that HLA changes appear to occur at a particular step in the progression from benign to most aggressive. Jiminez *et al* (Jiminez P, *et al.*, Microsatellite instability analysis in tumors with different mechanisms for total loss of HLA expression. *Cancer Immunol* Immunother 48:684-90, 2000) have analyzed 118 different tumors (68 colorectal, 34 laryngeal and 16 melanomas). The frequencies reported for total loss of HLA expression were 11% for colon, 18% for melanoma and 13 % for larynx. Thus, HLA class I expression is altered in a significant fraction of the tumor types, possibly as a reflection of immune pressure, or simply a reflection of the accumulation of pathological changes and alterations in diseased cells.

Immunotherapy in the context of HLA loss

A majority of the tumors express HLA class I, with a general tendency for the more severe alterations to be found in later stage and less differentiated tumors. This pattern is encouraging in the context of immunotherapy, especially considering that: 1) the relatively low sensitivity of

immunohistochemical techniques might underestimate HLA expression in tumors; 2) class I expression can be induced in tumor cells as a result of local inflammation and lymphokine release; and, 3) class I negative cells are sensitive to lysis by NK cells.

Accordingly, various embodiments of the present invention can be selected in view of the fact that there can be a degree of loss of HLA molecules, particularly in the context of neoplastic disease. For example, the treating physician can assay a patient's tumor to ascertain whether HLA is being expressed. If a percentage of tumor cells express no class I HLA, then embodiments of the present invention that comprise methods or compositions that elicit NK cell responses can be employed. As noted herein, such NK-inducing methods or composition can comprise a Flt3 ligand or ProGP which facilitate mobilization of dendritic cells, the rationale being that dendritic cells produce large amounts of IL-12. IL-12 can also be administered directly in either amino acid or nucleic acid form. It should be noted that compositions in accordance with the invention can be administered concurrently with NK cell-inducing compositions, or these compositions can be administered sequentially.

In the context of allele-specific HLA loss, a tumor retains class I expression and may thus escape NK cell recognition, yet still be susceptible to a CTL-based vaccine in accordance with the invention which comprises epitopes corresponding to the remaining HLA type. The concept here is analogous to embodiments of the invention that include multiple disease antigens to guard against mutations that yield loss of a specific antigen. Thus, one can simultaneously target multiple HLA specificities and epitopes from multiple disease-related antigens to prevent tumor escape by allele-specific loss as well as disease-related antigen loss. In addition, embodiments of the present invention can be combined with alternative therapeutic compositions and methods. Such alternative compositions and methods comprise, without limitation, radiation, cytotoxic pharmaceuticals, and/or compositions/methods that induce humoral antibody responses.

Moreover, it has been observed that expression of HLA can be upregulated by gamma IFN, which is commonly secreted by effector CTL, and that HLA class I expression can be induced in vivo by both alpha and beta IFN. Thus, embodiments of the invention can also comprise alpha, beta and/or gamma IFN to facilitate upregualtion of HLA.

IV.N. REPRIEVE PERIODS FROM THERAPIES THAT INDUCE SIDE EFFECTS: "Scheduled Treatment Interruptions or Drug Holidays"

Recent evidence has shown that certain patients infected with a pathogen, whom are initially treated with a therapeutic regimen to reduce pathogen load, have been able to maintain decreased pathogen load when removed from the therapeutic regimen, i.e., during a "drug holiday" (Rosenberg, E., *et al.*, Immune control of HIV-1 after early treatment of acute infection Nature 407:523-26, Sept. 28, 2000) As appreciated by those skilled in the art, many therapeutic regimens for both pathogens and cancer have numerous, often severe, side effects. During the drug holiday, the patient's immune system is keeping the disease in check. Methods for using compositions of the invention are used in the context of drug holidays for cancer and pathogenic infection.

For treatment of an infection, where therapies are not particularly immunosuppressive, compositions of the invention are administered concurrently with the standard therapy. During this period,

the patient's immune system is directed to induce responses against the epitopes comprised by the present inventive compositions. Upon removal from the treatment having side effects, the patient is primed to respond to the infectious pathogen should the pathogen load begin to increase. Composition of the invention can be provided during the drug holiday as well.

For patients with cancer, many therapies are immunosuppressive. Thus, upon achievement of a remission or identification that the patient is refractory to standard treatment, then upon removal from the immunosuppressive therapy, a composition in accordance with the invention is administered. Accordingly, as the patient's immune system reconstitutes, precious immune resources are simultaneously directed against the cancer. Composition of the invention can also be administered concurrently with an immunosuppressive regimen if desired.

IV.O. Kits

The peptide and nucleic acid compositions of this invention can be provided in kit form together with instructions for vaccine administration. Typically the kit would include desired peptide compositions in a container, preferably in unit dosage form and instructions for administration. An alternative kit would include a minigene construct with desired nucleic acids of the invention in a container, preferably in unit dosage form together with instructions for administration. Lymphokines such as IL-2 or IL-12 may also be included in the kit. Other kit components that may also be desirable include, for example, a sterile syringe, booster dosages, and other desired excipients.

IV.P. Overview

Epitopes in accordance with the present invention were successfully used to induce an immune response. Immune responses with these epitopes have been induced by administering the epitopes in various forms. The epitopes have been administered as peptides, as nucleic acids, and as viral vectors comprising nucleic acids that encode the epitope(s) of the invention. Upon administration of peptide-based epitope forms, immune responses have been induced by direct loading of an epitope onto an empty HLA molecule that is expressed on a cell, and via internalization of the epitope and processing via the HLA class I pathway; in either event, the HLA molecule expressing the epitope was then able to interact with and induce a CTL response. Peptides can be delivered directly or using such agents as liposomes. They can additionally be delivered using ballistic delivery, in which the peptides are typically in a crystalline form. When DNA is used to induce an immune response, it is administered either as naked DNA, generally in a dose range of approximately 1-5mg, or via the ballistic "gene gun" delivery, typically in a dose range of approximately 10-100 µg. The DNA can be delivered in a variety of conformations, *e.g.*, linear, circular *etc.* Various viral vectors have also successfully been used that comprise nucleic acids which encode epitopes in accordance with the invention.

Accordingly compositions in accordance with the invention exist in several forms. Embodiments of each of these composition forms in accordance with the invention have been successfully used to induce an immune response.

One composition in accordance with the invention comprises a plurality of peptides. This plurality or cocktail of peptides is generally admixed with one or more pharmaceutically acceptable

excipients. The peptide cocktail can comprise multiple copies of the same peptide or can comprise a mixture of peptides. The peptides can be analogs of naturally occurring epitopes. The peptides can comprise artificial amino acids and/or chemical modifications such as addition of a surface active molecule, *e.g.*, lipidation; acetylation, glycosylation, biotinylation, phosphorylation etc. The peptides can be CTL or HTL epitopes. In a preferred embodiment the peptide cocktail comprises a plurality of different CTL epitopes and at least one HTL epitope. The HTL epitope can be naturally or non-naturally (e.g., PADRE®, Epimmune Inc., San Diego, CA). The number of distinct epitopes in an embodiment of the invention is generally a whole unit integer from one through one hundred fifty (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or, 100).

An additional embodiment of a composition in accordance with the invention comprises a polypeptide multi-epitope construct, *i.e.*, a polyepitopic peptide. Polyepitopic peptides in accordance with the invention are prepared by use of technologies well-known in the art. By use of these known technologies, epitopes in accordance with the invention are connected one to another. The polyepitopic peptides can be linear or non-linear, *e.g.*, multivalent. These polyepitopic constructs can comprise artificial amino acids, spacing or spacer amino acids, flanking amino acids, or chemical modifications between adjacent epitope units. The polyepitopic construct can be a heteropolymer or a homopolymer. The polyepitopic constructs generally comprise epitopes in a quantity of any whole unit integer between 2-150 (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or, 100). The polyepitopic construct can comprise CTL and/or HTL epitopes. One or more of the epitopes in the construct can be modified, *e.g.*, by addition of a surface active material, *e.g.* a lipid, or chemically modified, *e.g.*, acetylation, *etc.* Moreover, bonds in the multiepitopic construct can be other than peptide bonds, *e.g.*, covalent bonds, ester or ether bonds, disulfide bonds, hydrogen bonds, ionic bonds *etc.*

Alternatively, a composition in accordance with the invention comprises construct which comprises a series, sequence, stretch, *etc.*, of amino acids that have homology to (*i.e.*, corresponds to or is contiguous with) to a native sequence. This stretch of amino acids comprises at least one subsequence of amino acids that, if cleaved or isolated from the longer series of amino acids, functions as an HLA class I or HLA class II epitope in accordance with the invention. In this embodiment, the peptide sequence is modified, so as to become a construct as defined herein, by use of any number of techniques known or to be provided in the art. The polyepitopic constructs can contain homology to a native sequence in any whole unit integer increment from 70-100%, e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or, 100 percent.

A further embodiment of a composition in accordance with the invention is an antigen presenting cell that comprises one or more epitopes in accordance with the invention. The antigen presenting cell can be a "professional" antigen presenting cell, such as a dendritic cell. The antigen

presenting cell can comprise the epitope of the invention by any means known or to be determined in the art. Such means include pulsing of dendritic cells with one or more individual epitopes or with one or more peptides that comprise multiple epitopes, by nucleic acid administration such as ballistic nucleic acid delivery or by other techniques in the art for administration of nucleic acids, including vector-based, *e.g.* viral vector, delivery of nucleic acids.

Further embodiments of compositions in accordance with the invention comprise nucleic acids that encode one or more peptides of the invention, or nucleic acids which encode a polyepitopic peptide in accordance with the invention. As appreciated by one of ordinary skill in the art, various nucleic acids compositions will encode the same peptide due to the redundancy of the genetic code. Each of these nucleic acid compositions falls within the scope of the present invention. This embodiment of the invention comprises DNA or RNA, and in certain embodiments a combination of DNA and RNA. It is to be appreciated that any composition comprising nucleic acids that will encode a peptide in accordance with the invention or any other peptide based composition in accordance with the invention, falls within the scope of this invention.

It is to be appreciated that peptide-based forms of the invention (as well as the nucleic acids that encode them) can comprise analogs of epitopes of the invention generated using priniciples already known, or to be known, in the art. Principles related to analoging are now known in the art, and are disclosed herein; moreover, analoging principles (heteroclitic analoging) are disclosed in co-pending application serial number U.S.S.N. 09/226,775 filed 6 January 1999. Generally the compositions of the invention are isolated or purified.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of non-critical parameters that can be changed or modified to yield alternative embodiments in accordance with the invention.

V. EXAMPLES

The following examples illustrate identification, selection, and use of immunogenic Class I and Class II peptide epitopes for inclusion in vaccine compositions.

Example 1. HLA Class I and Class II Binding Assays

The following example of peptide binding to HLA molecules demonstrates quantification of binding affinities of HLA class I and class II peptides. Binding assays can be performed with peptides that are either motif-bearing or not motif-bearing.

HLA class I and class II binding assays using purified HLA molecules were performed in accordance with disclosed protocols (*e.g.*, PCT publications WO 94/20127 and WO 94/03205; Sidney *et al.*, *Current Protocols in Immunology* 18.3.1 (1998); Sidney, *et al.*, *J. Immunol.* 154:247 (1995); Sette, *et al.*, *Mol. Immunol.* 31:813 (1994)). Briefly, purified MHC molecules (5 to 500nM) were incubated with various unlabeled peptide inhibitors and 1-10nM ^{125}I-radiolabeled probe peptides as described. Following incubation, MHC-peptide complexes were separated from free peptide by gel filtration and the fraction of

peptide bound was determined. Typically, in preliminary experiments, each MHC preparation was titered in the presence of fixed amounts of radiolabeled peptides to determine the concentration of HLA molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays were performed using these HLA concentrations.

Since under these conditions [label]<[HLA] and IC_{50}≥[HLA], the measured IC_{50} values are reasonable approximations of the true K_D values. Peptide inhibitors are typically tested at concentrations ranging from 120 μg/ml to 1.2 ng/ml, and are tested in two to four completely independent experiments. To allow comparison of the data obtained in different experiments, a relative binding figure is calculated for each peptide by dividing the IC_{50} of a positive control for inhibition by the IC_{50} for each tested peptide (typically unlabeled versions of the radiolabeled probe peptide). For database purposes, and inter-experiment comparisons, relative binding values are compiled. These values can subsequently be converted back into IC_{50} nM values by dividing the IC_{50} nM of the positive controls for inhibition by the relative binding of the peptide of interest. This method of data compilation has proven to be the most accurate and consistent for comparing peptides that have been tested on different days, or with different lots of purified MHC.

Binding assays as outlined above can be used to analyze supermotif and/or motif-bearing epitopes as, for example, described in Example 2.

Example 2. Identification of HLA Supermotif- and Motif-Bearing CTL Candidate Epitopes

Vaccine compositions of the invention may include multiple epitopes that comprise multiple HLA supermotifs or motifs to achieve broad population coverage. This example illustrates the identification of supermotif- and motif-bearing epitopes for the inclusion in such a vaccine composition. Calculation of population coverage is performed using the strategy described below.

Computer searches and algorithms for identification of supermotif and/or motif-bearing epitopes

The searches performed to identify the motif-bearing peptide sequences in Examples 2 and 5 employ protein sequence data for prostate cancer-associated antigens.

Computer searches for epitopes bearing HLA Class I or Class II supermotifs or motifs were performed as follows. All translated protein sequences were analyzed using a text string search software program, *e.g.*, MotifSearch 1.4 (D. Brown, San Diego) to identify potential peptide sequences containing appropriate HLA binding motifs; alternative programs are readily produced in accordance with information in the art in view of the motif/supermotif disclosure herein. Furthermore, such calculations can be made mentally.

Identified A2-, A3-, and DR-supermotif sequences were scored using polynomial algorithms to predict their capacity to bind to specific HLA-Class I or Class II molecules. These polynomial algorithms take into account both extended and refined motifs (that is, to account for the impact of different amino acids at different positions), and are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA molecule interactions can be approximated as a linear polynomial function of the type:

$$\text{“}\Delta G\text{”} = a_{1i} \times a_{2i} \times a_{3i} \ldots\ldots \times a_{ni}$$

where a_{ji} is a coefficient which represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. The crucial assumption of this method is that the effects at each position are essentially independent of each other (i.e., independent binding of individual side-chains). When residue j occurs at position i in the peptide, it is assumed to contribute a constant amount j_i to the free energy of binding of the peptide irrespective of the sequence of the rest of the peptide. This assumption is justified by studies from our laboratories that demonstrated that peptides are bound to MHC and recognized by T cells in essentially an extended conformation (data omitted herein).

The method of derivation of specific algorithm coefficients has been described in Gulukota *et al., J. Mol. Biol.* 267:1258-126, 1997; (*see* also Sidney *et al., Human Immunol.* 45:79-93, 1996; and Southwood *et al., J. Immunol.* 160:3363-3373, 1998). Briefly, for all i positions, anchor and non-anchor alike, the geometric mean of the average relative binding (ARB) of all peptides carrying j is calculated relative to the remainder of the group, and used as the estimate of j_i. For Class II peptides, if multiple alignments are possible, only the highest scoring alignment is utilized, following an iterative procedure. To calculate an algorithm score of a given peptide in a test set, the ARB values corresponding to the sequence of the peptide are multiplied. If this product exceeds a chosen threshold, the peptide is predicted to bind. Appropriate thresholds are chosen as a function of the degree of stringency of prediction desired.

Selection of HLA-A2 supertype cross-reactive peptides

The complete protein sequences of the prostate cancer-associated antigens PAP, PSA, PSM, and hK2 were obtained from GenBank and scanned, utilizing motif identification software, to identify 8-, 9-, 10-, and 11-mer sequences containing the HLA-A2-supermotif main anchor specificity.

HLA-A2 supermotif-bearing sequences are shown in Table VII. These sequences are then scored using the A2 algorithm and the peptides corresponding to the positive-scoring sequences are synthesized and tested for their capacity to bind purified HLA-A*0201 molecules *in vitro* (HLA-A*0201 is considered a prototype A2 supertype molecule).

Examples of peptides that were identified that bind to HLA-A*0201 with IC_{50} values ≤500 nM are shown in Tables XXII and XXIII. These peptides were then tested for the capacity to bind to additional A2-supertype molecules (A*0202, A*0203, A*0206, and A*6802). Peptides that bind to at least three of the five A2-supertype alleles tested are deemed A2-supertype cross-reactive binders. Preferred peptides bind at an affinity equal to or less than 500 nM to three or more HLA-A2 supertype molecules. Examples of such peptides are set out in Table XXIII. (Due to the homology described above, a number of CTL and HTL epitopes are represented in both the PSA and hK2 antigens. This is represented in Tables XXIII and XXIV by the headings source and alternate source.)

Selection of HLA-A3 supermotif-bearing epitopes

The protein sequences scanned above were also examined for the presence of peptides with the HLA-A3-supermotif primary anchors using methodology similar to that performed to identify HLA-A2 supermotif-bearing epitopes.

Peptides corresponding to the supermotif-bearing sequences are then synthesized and tested for binding to HLA-A*0301 and HLA-A*1101 molecules, the two most prevalent A3-supertype alleles. The peptides that are found to bind one of the two alleles with binding affinities of ≤500 nM, preferably ≤ 200 nM, are then tested for binding cross-reactivity to the other common A3-supertype alleles (A*3101, A*3301, and A*6801) to identify those that can bind at least three of the five HLA-A3-supertype molecules tested.

Selection of HLA-B7 supermotif bearing epitopes

The same target antigen protein sequences were also analyzed to identify HLA-B7-supermotif-bearing sequences. The corresponding peptides are then synthesized and tested for binding to HLA-B*0702, the most common B7-supertype allele (*i.e.*, the prototype B7 supertype allele). Those peptides that bind B*0702 with IC_{50} of ≤500 nM, preferably ≤ 200 nM, are then tested for binding to other common B7-supertype molecules (B*3501, B*5101, B*5301, and B*5401) to identify those peptides that are capable of binding to three or more of the five B7-supertype alleles tested.

Selection of A1 and A24 motif-bearing epitopes

To further increase population coverage, HLA-A1 and -A24 epitopes can also be incorporated into vaccine constructs. An analysis of the protein sequence data from the target antigens utilized above was performed to identify HLA-A1- and A24-motif-containing sequences. Peptides are then synthesized and tested for binding.

Peptides that bear other supermotifs and/or motifs can be assessed for binding or cross-reactive binding in an analogous manner.

Example 3. Confirmation of Immunogenicity

Cross-reactive candidate CTL A2-supermotif-bearing peptides that are identified as described in Example 2 were selected for *in vitro* immunogenicity testing. Examples of immunogenic HLA-A2 cross-reactive binding peptides that bind to at least 3/5 HLA-A2 supertype family members at an IC_{50} of 200 nM or less are shown in Table XXIV. Testing was performed using the following methodology:

Target Cell Lines for Cellular Screening:

The .221A2.1 cell line, produced by transferring the HLA-A2.1 gene into the HLA-A, -B, -C null mutant human B-lymphoblastoid cell line 721.221, is used as the peptide-loaded target to measure activity of HLA-A2.1-restricted CTL. This cell line is grown in RPMI-1640 medium supplemented with antibiotics, sodium pyruvate, nonessential amino acids and 10% (v/v) heat inactivated FCS. Cells that express an antigen of interest, or transfectants comprising the gene encoding the antigen of interest, can be used as target cells to test the ability of peptide-specific CTLs to recognize endogenous antigen.

Primary CTL Induction Cultures:

Generation of Dendritic Cells (DC): PBMCs are thawed in RPMI with 30 μg/ml DNAse, washed twice and resuspended in complete medium (RPMI-1640 plus 5% AB human serum, non-essential amino acids, sodium pyruvate, L-glutamine and penicillin/strpetomycin). The monocytes are purified by plating 10×10^6 PBMC/well in a 6-well plate. After 2 hours at 37°C, the non-adherent cells are removed by gently shaking the plates and aspirating the supernatants. The wells are washed a total of three times with 3 ml RPMI to remove most of the non-adherent and loosely adherent cells. Three ml of complete medium containing 50 ng/ml of GM-CSF and 1,000 U/ml of IL-4 are then added to each well. TNFα is added to the DCs on day 6 at 75 ng/ml and the cells are used for CTL induction cultures on day 7.

Induction of CTL with DC and Peptide: CD8+ T-cells are isolated by positive selection with Dynal immunomagnetic beads (Dynabeads® M-450) and the detacha-bead® reagent. Typically about $200\text{-}250 \times 10^6$ PBMC are processed to obtain 24×10^6 $CD8^+$ T-cells (enough for a 48-well plate culture). Briefly, the PBMCs are thawed in RPMI with 30μg/ml DNAse, washed once with PBS containing 1% human AB serum and resuspended in PBS/1% AB serum at a concentration of 20×10^6cells/ml. The magnetic beads are washed 3 times with PBS/AB serum, added to the cells (140μl beads/20×10^6 cells) and incubated for 1 hour at 4°C with continuous mixing. The beads and cells are washed 4x with PBS/AB serum to remove the nonadherent cells and resuspended at 100×10^6 cells/ml (based on the original cell number) in PBS/AB serum containing 100μl/ml detacha-bead® reagent and 30μg/ml DNAse. The mixture is incubated for 1 hour at room temperature with continuous mixing. The beads are washed again with PBS/AB/DNAse to collect the CD8+ T-cells. The DC are collected and centrifuged at 1300 rpm for 5-7 minutes, washed once with PBS with 1% BSA, counted and pulsed with 40μg/ml of peptide at a cell concentration of $1\text{-}2 \times 10^6$/ml in the presence of 3μg/ml β_2- microglobulin for 4 hours at 20°C. The DC are then irradiated (4,200 rads), washed 1 time with medium and counted again.

Setting up induction cultures: 0.25 ml cytokine-generated DC (@1×10^5 cells/ml) are co-cultured with 0.25ml of CD8+ T-cells (@2×10^6 cell/ml) in each well of a 48-well plate in the presence of 10 ng/ml of IL-7. Recombinant human IL10 is added the next day at a final concentration of 10 ng/ml and rhuman IL2 is added 48 hours later at 10IU/ml.

Restimulation of the induction cultures with peptide-pulsed adherent cells: Seven and fourteen days after the primary induction the cells are restimulated with peptide-pulsed adherent cells. The PBMCS are thawed and washed twice with RPMI and DNAse. The cells are resuspended at 5×10^6 cells/ml and irradiated at ~4200 rads. The PBMCs are plated at 2×10^6 in 0.5ml complete medium per well and incubated for 2 hours at 37°C. The plates are washed twice with RPMI by tapping the plate gently to remove the nonadherent cells and the adherent cells pulsed with 10μg/ml of peptide in the presence of 3 μg/ml β_2 microglobulin in 0.25ml RPMI/5%AB per well for 2 hours at 37°C. Peptide solution from each well is aspirated and the wells are washed once with RPMI. Most of the media is aspirated from the induction cultures (CD8+ cells) and brought to 0.5 ml with fresh media. The cells are then transferred to the wells containing the peptide-pulsed adherent cells. Twenty four hours later rhuman IL10 is added at a final concentration of 10ng/ml and rhuman IL2 is added the next day and again 2-3 days later at 50IU/ml (Tsai *et al.*, *Critical Reviews in Immunology* 18(1-2):65-75, 1998). Seven days later the cultures are assayed for CTL activity in a ^{51}Cr release assay. In some experiments the cultures are assayed for peptide-

specific recognition in the *in situ* IFNγ ELISA at the time of the second restimulation followed by assay of endogenous recognition 7 days later. After expansion, activity is measured in both assays for a side by side comparison.

Measurement of CTL lytic activity by ^{51}Cr release.

Seven days after the second restimulation, cytotoxicity is determined in a standard (5hr) ^{51}Cr release assay by assaying individual wells at a single E:T. Peptide-pulsed targets are prepared by incubating the cells with 10μg/ml peptide overnight at 37°C.

Adherent target cells are removed from culture flasks with trypsin-EDTA. Target cells are labelled with 200μCi of ^{51}Cr sodium chromate (Dupont, Wilmington, DE) for 1 hour at 37°C. Labelled target cells are resuspended at 10^6 per ml and diluted 1:10 with K562 cells at a concentration of $3.3x10^6$/ml (an NK-sensitive erythroblastoma cell line used to reduce non-specific lysis). Target cells (100 μl) and 100μl of effectors are plated in 96 well round-bottom plates and incubated for 5 hours at 37°C. At that time, 100 μl of supernatant are collected from each well and percent lysis is determined according to the formula: [(cpm of the test sample- cpm of the spontaneous ^{51}Cr release sample)/(cpm of the maximal ^{51}Cr release sample- cpm of the spontaneous ^{51}Cr release sample)] x 100. Maximum and spontaneous release are determined by incubating the labelled targets with 1% Trition X-100 and media alone, respectively. A positive culture is defined as one in which the specific lysis (sample- background) is 10% or higher in the case of individual wells and is 15% or more at the 2 highest E:T ratios when expanded cultures are assayed.

***In situ* Measurement of Human γIFN Production as an Indicator of Peptide-specific and Endogenous Recognition**

Immulon 2 plates are coated with mouse anti-human IFNγ monoclonal antibody (4 μg/ml 0.1M $NaHCO_3$, pH8.2) overnight at 4°C. The plates are washed with Ca^{2+}, Mg^{2+}-free PBS/0.05% Tween 20 and blocked with PBS/10% FCS for 2 hours, after which the CTLs (100 μl/well) and targets (100 μl/well) are added to each well, leaving empty wells for the standards and blanks (which received media only). The target cells, either peptide-pulsed or endogenous targets, are used at a concentration of $1x10^6$ cells/ml. The plates are incubated for 48 hours at 37°C with 5% CO_2.

Recombinant human IFNγ is added to the standard wells starting at 400 pg or 1200pg/100μl/well and the plate incubated for 2 hours at 37°C. The plates are washed and 100 μl of biotinylated mouse anti-human IFNγ monoclonal antibody (2μg/ml in PBS/3%FCS/0.05% Tween 20) are added and incubated for 2 hours at room temperature. After washing again, 100 μl HRP-streptavidin (1:4000) are added and the plates incubated for 1 hour at room temperature. The plates are then washed 6x with wash buffer, 100μl/well developing solution (TMB 1:1) are added, and the plates allowed to develop for 5-15 minutes. The reaction is stopped with 50 μl/well 1M H_3PO_4 and read at OD450. A culture is considered positive if it measured at least 50 pg of IFNγ/well above background and is twice the background level of expression.

CTL Expansion. Those cultures that demonstrate specific lytic activity against peptide-pulsed targets and/or tumor targets are expanded over a two week period with anti-CD3. Briefly, $5x10^4$ CD8+ cells are added to a T25 flask containing the following: $1x10^6$ irradiated (4,200 rad) PBMC

(autologous or allogeneic) per ml, $2x10^5$ irradiated (8,000 rad) EBV- transformed cells per ml, and OKT3 (anti-CD3) at 30ng per ml in RPMI-1640 containing 10% (v/v) human AB serum, non-essential amino acids, sodium pyruvate, 25μM 2-mercaptoethanol, L-glutamine and penicillin/streptomycin. Rhuman IL2 is added 24 hours later at a final concentration of 200IU/ml and every 3 days thereafter with fresh media at 50IU/ml. The cells are split if the cell concentration exceeded $1x10^6$/ml and the cultures are assayed between days 13 and 15 at E:T ratios of 30, 10, 3 and 1:1 in the ^{51}Cr release assay or at $1x10^6$/ml in the *in situ* IFNγ assay using the same targets as before the expansion.

Cultures are expanded in the absence of anti-$CD3^+$ as follows. Those cultures that demonstrate specific lytic activity against peptide and endogenous targets are selected and $5x10^4$ $CD8^+$ cells are added to a T25 flask containing the following: $1x10^6$ autologous PBMC per ml which have been peptide-pulsed with 10μg/ml peptide for 2 hours at 37°C and irradiated (4,200 rad); $2x10^5$ irradiated (8,000 rad) EBV-transformed cells per ml RPMI-1640 containing 10%(v/v) human AB serum, non-essential AA, sodium pyruvate, 25mM 2-ME, L-glutamine and gentamicin.

Immunogenicity of A2 supermotif-bearing peptides

A2-supermotif cross-reactive binding peptides were tested in the cellular assay for the ability to induce peptide-specific CTL in normal individuals. In this analysis, a peptide is considered to be an epitope if it induces peptide-specific CTLs in at least 2 donors (unless otherwise noted) and preferably, also recognizes the endogenously expressed peptide. Examples of immunogenic peptides are shown in Table XXIV.

Immunogenicity is additionally confirmed using PBMCs isolated from cancer patients. Briefly, PBMCs are isolated from patients with prostate cancer, re-stimulated with peptide-pulsed monocytes and assayed for the ability to recognize peptide-pulsed target cells as well as transfected cells endogenously expressing the antigen.

*Evaluation of A*03/A11 immunogenicity*

HLA-A3 supermotif-bearing cross-reactive binding peptides are also evaluated for immunogenicity using methodology analogous for that used to evaluate the immunogenicity of the HLA-A2 supermotif peptides.

Evaluation of B7 immunogenicity

Immunogenicity screening of the B7-supertype cross-reactive binding peptides identified in Example 2 are evaluated in a manner analogous to the evaluation of A2-and A3-supermotif-bearing peptides.

Peptides bearing other supermotifs and/or motifs, *e.g.*, HLA-A1, HLA-a24 *etc.* are also evaluated using similar methodology

Example 4. Implementation of the Extended Supermotif to Improve the Binding Capacity of Native Epitopes by Creating Analogs

HLA motifs and supermotifs (comprising primary and/or secondary residues) are useful in the identification and preparation of highly cross-reactive native peptides, as demonstrated herein. Moreover, the definition of HLA motifs and supermotifs also allows one to engineer highly cross-reactive epitopes by identifying residues within a native peptide sequence which can be analoged, or "fixed" to confer upon the peptide certain characteristics, *e.g.* greater cross-reactivity within the group of HLA molecules that comprise a supertype, and/or greater binding affinity for some or all of those HLA molecules. Examples of analog peptides that exhibit modulated binding affinity are set forth in this example.

Analoging at Primary Anchor Residues

Peptide engineering strategies were implemented to further increase the cross-reactivity of the epitopes identified above (*see, e.g.*, Table XXIII). On the basis of the data disclosed, *e.g.*, in related and co-pending U.S.S.N 09/226,775, the main anchors of A2-supermotif-bearing peptides are altered, for example, to introduce a preferred L, I, V, or M at position 2, and I or V at the C-terminus.

Peptides that exhibit at least weak A*0201 binding (IC_{50} of 5000 nM or less), and carrying suboptimal anchor residues at either position 2, the C-terminal position, or both, can be fixed by introducing canonical substitutions (typically L at position 2 and V at the C-terminus). Those analoged peptides that show at least a three-fold increase in A*0201 binding and bind with an IC_{50} of 500 nM, or preferably 200 nM, or less are then tested for A2 cross-reactive binding along with their wild-type (WT) counterparts. Analoged peptides that bind at least three of the five A2 supertype alleles are then selected for cellular screening analysis.

Additionally, the selection of analogs for cellular screening analysis is further restricted by the capacity of the WT parent peptide to bind at least weakly, *i.e.*, bind at an IC_{50} of 5000nM or less, to three of more A2 supertype alleles. The rationale for this requirement is that the WT peptides must be present endogenously in sufficient quantity to be biologically relevant. Analoged peptides have been shown to have increased immunogenicity and cross-reactivity by T cells specific for the WT epitope (*see, e.g.*, Parkhurst *et al.*, *J. Immunol.* 157:2539, 1996; and Pogue *et al.*, *Proc. Natl. Acad. Sci. USA* 92:8166, 1995).

In the cellular screening of these peptide analogs, it is important to demonstrate that analog-specific CTLs are also able to recognize the wild-type peptide and, when possible, tumor targets that endogenously express the epitope.

Peptides that were analoged at primary anchor residues, generally by adding a preferred residue at a primary anchor position, were synthesized and assessed for enhanced binding to A*0201 and/or enhanced cross-reactive binding. Examples of analoged peptides that exhibit increased binding and/or cross-reactivity are shown in Table XXIII.

Analogs exhibiting altered binding characteristics are then selected for cellular screening studies. Examples are shown in Table XXIV.

Using methodology similar to that used to develop HLA-A2 analogs, analogs of HLA-A3 and HLA-B7 supermotif-bearing epitopes are also generated. Analogous strategies can be used for peptides bearing other supermotifs/motifs as well. For example, peptides binding at least weakly to 3/5 of the A3-supertype molecules may be engineered at primary anchor residues to possess a preferred residue (V, S, M, or A) at position 2. The analog peptides are then tested for the ability to bind A*03 and A*11 (prototype A3 supertype alleles). Those peptides that demonstrate ≤ 500 nM binding capacity, often ≤ 200 nM binding values, are then tested for A3-supertype cross-reactivity. B7 supermotif-bearing peptides may, for example, be engineered to possess a preferred residue (V, I, L, or F) at the C-terminal primary anchor position, as demonstrated by Sidney *et al.* (*J. Immunol.* 157:3480-3490, 1996) and tested for binding to B7 supertype alleles.

Analoging at Secondary Anchor Residues

Moreover, HLA supermotifs are of value in engineering highly cross-reactive peptides and/or peptides that bind HLA molecules with increased affinity by identifying particular residues at secondary anchor positions that are associated with such properties. For example, the binding capacity of a B7 supermotif-bearing peptide representing a discreet single amino acid substitution at position 1 can be analyzed. A peptide can, for example, be analoged to substitute L with F at position 1 and subsequently be evaluated for increased binding affinity/ and or increased cross-reactivity. This procedure will identify analoged peptides with modulated binding affinity.

Engineered analogs with sufficiently improved binding capacity or cross-reactivity are tested for immunogenicity as above.

Other analoging strategies

Another form of peptide analoging, unrelated to the anchor positions, involves the substitution of a cysteine with α-amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Subtitution of α-amino butyric acid for cysteine not only alleviates this problem, but has been shown to improve binding and crossbinding capabilities in some instances (*see, e.g.*, the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999)..

In conclusion, these data demonstrate that by the use of even single amino acid substitutions, it is possible to increase the binding affinity and/or cross-reactivity of peptide ligands for HLA supertype molecules.

Example 5. Identification of peptide epitope sequences with HLA-DR binding motifs

Peptide epitopes bearing an HLA class II supermotif or motif may also be identified as outlined below using methodology similar to that described in Examples 1-3.

Selection of HLA-DR-supermotif-bearing epitopes

To identify HLA class II HTL epitopes, the prostate cancer-associate antigen protein sequences were analyzed for the presence of sequences bearing an HLA-DR-motif or supermotif.

Specifically, 15-mer sequences are selected comprising a DR-supermotif, further comprising a 9-mer core, and three-residue N- and C-terminal flanking regions (15 amino acids total).

Protocols for predicting peptide binding to DR molecules have been developed (Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). These protocols, specific for individual DR molecules, allow the scoring, and ranking, of 9-mer core regions. Each protocol not only scores peptide sequences for the presence of DR-supermotif primary anchors (i.e., at position 1 and position 6) within a 9-mer core, but additionally evaluates sequences for the presence of secondary anchors. Using allele specific selection tables (*see, e.g.*, Southwood *et al.*, *ibid.*), it has been found that these protocols efficiently select peptide sequences with a high probability of binding a particular DR molecule. Additionally, it has been found that performing these protocols in tandem, specifically those for DR1, DR4w4, and DR7, can efficiently select DR cross-reactive peptides.

The prostate antigen-derived peptides identified above are tested for their binding capacity to various common HLA-DR molecules. All peptides are initially tested for binding to the DR molecules in the primary panel: DR1, DR4w4, and DR7. Peptides binding at least 2 of these 3 DR molecules with an IC_{50} value of 1000 nM or less, were then tested for binding to DR5*0101, DRB1*1501, DRB1*1101, DRB1*0802, and DRB1*1302. Peptides were considered to be cross-reactive DR supertype binders if they bound at an IC_{50} value of 1000 nM or less to at least 5 of the 8 alleles tested.

Following the strategy outlined above DR supermotif-bearing sequences were identified within the prostate antigen protein sequence. Generally, these sequences are then scored for the combined DR 1-4-7 algorithms. The postive-scoring peptides are synthesized and tested for binding to HLA-DRB1*0101, DRB1*0401, DRB1*0701. Those that bind at least 2 of the 3 alleles are then tested for binding to secondary DR supertype alleles: DRB5*0101, DRB1*1501, DRB1*1101, DRB1*0802, and DRB1*1302.

Selection of DR3 motif peptides

Because HLA-DR3 is an allele that is prevalent in Caucasian, Black, and Hispanic populations, DR3 binding capacity is an important criterion in the selection of HTL epitopes. However, data generated previously indicated that DR3 only rarely cross-reacts with other DR alleles (Sidney *et al.*, *J. Immunol.* 149:2634-2640, 1992; Geluk *et al.*, *J. Immunol.* 152:5742-5748, 1994; Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). This is not entirely surprising in that the DR3 peptide-binding motif appears to be distinct from the specificity of most other DR alleles. For maximum efficiency in developing vaccine candidates it would be desirable for DR3 motifs to be clustered in proximity with DR supermotif regions. Thus, peptides shown to be candidates may also be assayed for their DR3 binding capacity. However, in view of the distinct binding specificity of the DR3 motif, peptides binding only to DR3 can also be considered as candidates for inclusion in a vaccine formulation.

To efficiently identify peptides that bind DR3, the PSA, PSM, PAP, and hK2 protein sequences were analyzed for sequences carrying one of the two DR3 specific binding motifs (Table III) reported by Geluk *et al.* (*J. Immunol.* 152:5742-5748, 1994). The corresponding peptides are then synthesized and tested for the ability to bind DR3 with an affinity of 1000 nM or better, *i.e.*, less than 1000 nM.

Additionally, the DR3 binders are also tested for binding to the DR supertype alleles. Conversely, the DR supertype cross-reactive binding peptides are also tested for DR3 binding capacity.

DR3 binding epitopes identified in this manner are then included in vaccine compositions with DR supermotif-bearing peptide epitopes.

Similarly to the case of HLA class I motif-bearing peptides, the class II motif-bearing peptides are analoged to improve affinity or cross-reactivity. For example, aspartic acid at position 4 of the 9-mer core sequence is an optimal residue for DR3 binding, and substitution for that residue often improves DR 3 binding.

For example, a number of HLA-DR supermotif and DR-3 motif-bearing prostate antigen-associated sequences have been identified. The number in each category is summarized in Table XXV.

Example 6. Immunogenicity of HTL epitopes

This example determines immunogenic DR supermotif- and DR3 motif-bearing epitopes among those identified using the methodology in Example 5.

Immunogenicity of HTL epitopes are evaluated in a manner analogous to the determination of immunogenicity of CTL epitopes by assessing the ability to stimulate HTL responses and/or by using appropriate transgenic mouse models. Immunogenicity is determined by screening for: 1.) *in vitro* primary induction using normal PBMC or 2.) recall responses from cancer patient PBMCs.

Example 7. Calculation of phenotypic frequencies of HLA-supertypes in various ethnic backgrounds to determine breadth of population coverage

This example illustrates the assessment of the breadth of population coverage of a vaccine composition comprised of multiple epitopes comprising multiple supermotifs and/or motifs.

In order to analyze population coverage, gene frequencies of HLA alleles were determined. Gene frequencies for each HLA allele were calculated from antigen or allele frequencies utilizing the binomial distribution formulae gf=1-(SQRT(1-af)) (see, *e.g.*, Sidney *et al.*, *Human Immunol.* 45:79-93, 1996). To obtain overall phenotypic frequencies, cumulative gene frequencies were calculated, and the cumulative antigen frequencies derived by the use of the inverse formula [af=1-(1-Cgf)^2].

Where frequency data was not available at the level of DNA typing, correspondence to the serologically defined antigen frequencies was assumed. To obtain total potential supertype population coverage no linkage disequilibrium was assumed, and only alleles confirmed to belong to each of the supertypes were included (minimal estimates). Estimates of total potential coverage achieved by inter-loci combinations were made by adding to the A coverage the proportion of the non-A covered population that could be expected to be covered by the B alleles considered (*e.g.*, total=A+B*(1-A)). Confirmed members of the A3-like supertype are A3, A11, A31, A*3301, and A*6801. Although the A3-like supertype may also include A34, A66, and A*7401, these alleles were not included in overall frequency calculations. Likewise, confirmed members of the A2-like supertype family are A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802, and A*6901. Finally, the B7-like supertype-confirmed alleles are: B7, B*3501-03, B51, B*5301, B*5401, B*5501-2, B*5601, B*6701, and B*7801 (potentially also B*1401, B*3504-06, B*4201, and B*5602).

Population coverage achieved by combining the A2-, A3- and B7-supertypes is approximately 86% in five major ethnic groups (see Table XXI). Coverage may be extended by including peptides bearing the A1 and A24 motifs. On average, A1 is present in 12% and A24 in 29% of the population across five different major ethnic groups (Caucasian, North American Black, Chinese, Japanese, and Hispanic). Together, these alleles are represented with an average frequency of 39% in these same ethnic populations. The total coverage across the major ethnicities when A1 and A24 are combined with the coverage of the A2-, A3- and B7-supertype alleles is >95%. An analogous approach can be used to estimate population coverage achieved with combinations of class II motif-bearing epitopes.

Example 8. Recognition Of Generation Of Endogenous Processed Antigens After Priming

This example determines that CTL induced by native or analogued peptide epitopes identified and selected as described in Examples 1-6 recognize endogenously synthesized, *i.e.*, native antigens, using a transgenic mouse model.

Effector cells isolated from transgenic mice that are immunized with peptide epitopes (as described, *e.g.*, in Wentworth et al., *Mol. Immunol.* 32:603, 1995), for example HLA-A2 supermotif-bearing epitopes, are re-stimulated *in vitro* using peptide-coated stimulator cells. Six days later, effector cells are assayed for cytotoxicity and the cell lines that contain peptide-specific cytotoxic activity are further re-stimulated. An additional six days later, these cell lines are tested for cytotoxic activity on ^{51}Cr labeled Jurkat-A2.1/K^b target cells in the absence or presence of peptide, and also tested on ^{51}Cr labeled target cells bearing the endogenously synthesized antigen, *i.e.* prostate tumor cells or cells that are stably transfected with TAA expression vectors.

The result will demonstrate that CTL lines obtained from animals primed with peptide epitope recognize endogenously synthesized antigen. The choice of transgenic mouse model to be used for such an analysis depends upon the epitope(s) that is being evaluated. In addition to HLA-A*0201/K^b transgenic mice, several other transgenic mouse models including mice with human A11, which may also be used to evaluate A3 epitopes, and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed, which may be used to evaluate HTL epitopes.

Example 9. Activity Of CTL-HTL Conjugated Epitopes In Transgenic Mice

This example illustrates the induction of CTLs and HTLs in transgenic mice by use of a tumor associated antigen CTL/HTL peptide conjugate whereby the vaccine composition comprises peptides to be administered to a cancer patient. The peptide composition can comprise multiple CTL and/or HTL epitopes and further, can comprise epitopes selected from multiple-tumor associated antigens. The epitopes are identified using methodology as described in Examples 1-6 This analysis demonstrates the enhanced immunogenicity that can be achieved by inclusion of one or more HTL epitopes in a vaccine composition. Such a peptide composition can comprise an HTL epitope conjugated to a preferred CTL epitope containing, for example, at least one CTL epitope selected from Table XXIII, or other analogs of that epitope. The peptides may be lipidated, if desired.

Immunization procedures: Immunization of transgenic mice is performed as described (Alexander *et al.*, *J. Immunol.* 159:4753-4761, 1997). For example, A2/K^b mice, which are transgenic for the human HLA A2.1 allele and are useful for the assessment of the immunogenicity of HLA-A*0201 motif- or HLA-A2 supermotif-bearing epitopes, are primed subcutaneously (base of the tail) with a 0.1 ml of peptide in Incomplete Freund's Adjuvant, or if the peptide composition is a lipidated CTL/HTL conjugate, in DMSO/saline or if the peptide composition is a polypeptide, in PBS or Incomplete Freund's Adjuvant. Seven days after priming, splenocytes obtained from these animals are restimulated with syngenic irradiated LPS-activated lymphoblasts coated with peptide.

The target cells for peptide-specific cytotoxicity assays are Jurkat cells transfected with the HLA-A2.1/K^b chimeric gene (*e.g.*, Vitiello *et al.*, *J. Exp. Med.* 173:1007, 1991).

In vitro CTL activation: One week after priming, spleen cells ($30x10^6$ cells/flask) are co-cultured at 37°C with syngeneic, irradiated (3000 rads), peptide coated lymphoblasts ($10x10^6$ cells/flask) in 10 ml of culture medium/T25 flask. After six days, effector cells are harvested and assayed for cytotoxic activity.

Assay for cytotoxic activity: Target cells (1.0 to $1.5x10^6$) are incubated at 37°C in the presence of 200 µl of ^{51}Cr. After 60 minutes, cells are washed three times and resuspended in medium. Peptide is added where required at a concentration of 1 µg/ml. For the assay, 10^4 ^{51}Cr-labeled target cells are added to different concentrations of effector cells (final volume of 200 µl) in U-bottom 96-well plates. After a 6 hour incubation period at 37°C, a 0.1 ml aliquot of supernatant is removed from each well and radioactivity is determined in a Micromedic automatic gamma counter. The percent specific lysis is determined by the formula: percent specific release = 100 x (experimental release - spontaneous release)/(maximum release - spontaneous release). To facilitate comparison between separate CTL assays run under the same conditions, % ^{51}Cr release data is expressed as lytic units/10^6 cells. One lytic unit is arbitrarily defined as the number of effector cells required to achieve 30% lysis of 10,000 target cells in a 6 hour ^{51}Cr release assay. To obtain specific lytic units/10^6, the lytic units/10^6 obtained in the absence of peptide is subtracted from the lytic units/10^6 obtained in the presence of peptide. For example, if 30% ^{51}Cr release is obtained at the effector (E): target (T) ratio of 50:1 (i.e., $5x10^5$ effector cells for 10,000 targets) in the absence of peptide and 5:1 (i.e., $5x10^4$ effector cells for 10,000 targets) in the presence of peptide, the specific lytic units would be: $[(1/50,000)-(1/500,000)] \times 10^6 = 18$ LU.

The results are analyzed to assess the magnitude of the CTL responses of animals injected with the immmunogenic CTL/HTL conjugate vaccine preparation. The magnitude and frequency of the response can also be compared to the the CTL response achieved using the CTL epitopes by themselves. Analyses similar to this may be performed to evaluate the immunogenicity of peptide conjugates containing multiple CTL epitopes and/or multiple HTL epitopes. In accordance with these procedures it is found that a CTL response is induced, and concomitantly that an HTL response is induced upon administration of such compositions.

Example 10. Selection of CTL and HTL epitopes for inclusion in a cancer vaccine.

This example illustrates the procedure for the selection of peptide epitopes for vaccine compositions of the invention. The peptides in the composition can be in the form of a nucleic acid

sequence, either single or one or more sequences (*i.e.*, minigene) that encodes peptide(s), or may be single and/or polyepitopic peptides.

The following principles are utilized when selecting an array of epitopes for inclusion in a vaccine composition. Each of the following principles is balanced in order to make the selection.

Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For example, a vaccine can include 3-4 epitopes that come from at least one prostate cancer-associated antigen. Epitopes from one prostate cancer-associated antigen can be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs as described, *e.g.*, in Example 15.

Epitopes are preferably selected that have a binding affinity (IC_{50}) of 500 nM or less, often 200 nM or less, for an HLA class I molecule, or for a class II molecule, 1000 nM or less.

Sufficient supermotif bearing peptides, or a sufficient array of allele-specific motif bearing peptides, are selected to give broad population coverage. For example, epitopes are selected to provide at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess breadth, or redundancy, of population coverage.

When selecting epitopes from cancer-related antigens it is often preferred to select analogs because the patient may have developed tolerance to the native epitope.

When creating a polyepitopic composition, *e.g.* a minigene, it is typically desirable to generate the smallest peptide possible that encompasses the epitopes of interest, although spacers or other flanking sequences can also be incorporated. The principles employed are often similar as those employed when selecting a peptide comprising nested epitopes. Additionally, however, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide sequence encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is a potential HLA binding epitope, as predicted, *e.g.*, by motif analysis. Junctional epitopes are generally to be avoided because the recipient may bind to an HLA molecule and generate an immune response to that epitope, which is not present in a native protein sequence.

A vaccine composition comprised of selected peptides, when administered, is safe, efficacious, and elicits an immune response that results in tumor cell killing and reduction of tumor size or mass.

Example 11. Construction of Minigene Multi-Epitope DNA Plasmids

This example provides general guidance for the construction of a minigene expression plasmid. Minigene plasmids may, of course, contain various configurations of CTL and/or HTL epitopes or epitope analogs as described herein. Examples of the construction and evaluation of expression plasmids are described, for example, in co-pending U.S.S.N. 09/311,784 filed 5/13/99.

A minigene expression plasmid may include multiple CTL and HTL peptide epitopes. In this example, HLA-A2, -A3, -B7 supermotif-bearing peptide epitopes and HLA-A1 and -A24 motif-bearing peptide epitopes are used in conjunction with DR supermotif-bearing epitopes and/or DR3 epitopes. HLA class I supermotif or motif-bearing peptide epitopes derived from multiple prostate cancer-associated

antigens are selected such that multiple supermotifs/motifs are represented to ensure broad population coverage. Similarly, HLA class II epitopes are selected from multiple prostate cancer-associated antigens to provide broad population coverage, *i.e.* both HLA DR-1-4-7 supermotif-bearing epitopes and HLA DR-3 motif-bearing epitopes are selected for inclusion in the minigene construct. The selected CTL and HTL epitopes are then incorporated into a minigene for expression in an expression vector.

This example illustrates the methods to be used for construction of such a minigene-bearing expression plasmid. Other expression vectors that may be used for minigene compositions are available and known to those of skill in the art.

The minigene DNA plasmid contains a consensus Kozak sequence and a consensus murine kappa Ig-light chain signal sequence followed by CTL and/or HTL epitopes selected in accordance with principles disclosed herein. The sequence encodes an open reading frame fused to the Myc and His antibody epitope tag coded for by the pcDNA 3.1 Myc-His vector.

Overlapping oligonucleotides that can, for example, average about 70 nucleotides in length with 15 nucleotide overlaps, are synthesized and HPLC-purified. The oligonucleotides encode the selected peptide epitopes as well as appropriate linker nucleotides, Kozak sequence, and signal sequence. The final multiepitope minigene is assembled by extending the overlapping oligonucleotides in three sets of reactions using PCR. A Perkin/Elmer 9600 PCR machine is used and a total of 30 cycles are performed using the following conditions: 95°C for 15 sec, annealing temperature (5° below the lowest calculated Tm of each primer pair) for 30 sec, and 72°C for 1 min.

For example, a minigene can be prepared as follows. For a first PCR reaction, 5 µg of each of two oligonucleotides are annealed and extended: In an example using eight oligonucleotides, *i.e.*, four pairs of primers, oligonucleotides 1+2, 3+4, 5+6, and 7+8 are combined in 100 µl reactions containing *Pfu* polymerase buffer (1x= 10 mM KCL, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-chloride, pH 8.75, 2 mM $MgSO_4$, 0.1% Triton X-100, 100 µg/ml BSA), 0.25 mM each dNTP, and 2.5 U of *Pfu* polymerase. The full-length dimer products are gel-purified, and two reactions containing the product of 1+2 and 3+4, and the product of 5+6 and 7+8 are mixed, annealed, and extended for 10 cycles. Half of the two reactions are then mixed, and 5 cycles of annealing and extension carried out before flanking primers are added to amplify the full length product. The full-length product is gel-purified and cloned into pCR-blunt (Invitrogen) and individual clones are screened by sequencing.

Example 12. The plasmid construct and the degree to which it induces immunogenicity.

The degree to which a plasmid construct, for example a plasmid constructed in accordance with Example 11, is able to induce immunogenicity can be evaluated *in vitro* by testing for epitope presentation by APC following transduction or transfection of the APC with an epitope-expressing nucleic acid construct. Such a study determines "antigenicity" and allows the use of human APC. The assay determines the ability of the epitope to be presented by the APC in a context that is recognized by a T cell by quantifying the density of epitope-HLA class I complexes on the cell surface. Quantitation can be performed by directly measuring the amount of peptide eluted from the APC (*see, e.g.*, Sijts *et al., J. Immunol.* 156:683-692, 1996; Demotz *et al., Nature* 342:682-684, 1989); or the number of peptide-HLA class I complexes can be estimated by measuring the amount of lysis or lymphokine release induced by

infected or transfected target cells, and then determining the concentration of peptide necessary to obtained equivalent levels of lysis or lymphokine release (*see, e.g.*, Kageyama *et al.*, *J. Immunol.* 154:567-576, 1995).

Atlernatively, immunogenicity can be evaluated through *in vivo* injections into mice and subsequent *in vitro* assessment of CTL and HTL activity, which are analysed using cytotoxicity and proliferation assays, respectively, as detailed *e.g.*, in co-pending U.S.S.N. 09/311,784 filed 5/13/99 and Alexander *et al.*, *Immunity* 1:751-761, 1994.

For example, to assess the capacity of a DNA minigene construct (*e.g.*, a pMin minigene construct generated as decribed in U.S.S.N. 09/311,784) containing at least one HLA-A2 supermotif peptide to induce CTLs *in vivo*, HLA-A2.1/K^b transgenic mice, for example, are immunized intramuscularly with 100 μg of naked cDNA. As a means of comparing the level of CTLs induced by cDNA immunization, a control group of animals is also immunized with an actual peptide composition that comprises multiple epitopes synthesized as a single polypeptide as they would be encoded by the minigene.

Splenocytes from immunized animals are stimulated twice with each of the respective compositions (peptide epitopes encoded in the minigene or the polyepitopic peptide), then assayed for peptide-specific cytotoxic activity in a ^{51}Cr release assay. The results indicate the magnitude of the CTL response directed against the A2-restricted epitope, thus indicating the *in vivo* immunogenicity of the minigene vaccine and polyepitopic vaccine. It is, therefore, found that the minigene elicits immune responses directed toward the HLA-A2 supermotif peptide epitopes as does the polyepitopic peptide vaccine. A similar analysis is also performed using other HLA-A3 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A3 and HLA-B7 motif or supermotif epitopes.

To assess the capacity of a class II epitope encoding minigene to induce HTLs *in vivo*, DR transgenic mice, or for those epitope that cross react with the appropriate mouse MHC molecule, I-A^b-restricted mice, for example, are immunized intramuscularly with 100 μg of plasmid DNA. As a means of comparing the level of HTLs induced by DNA immunization, a group of control animals is also immunized with an actual peptide composition emulsified in complete Freund's adjuvant. CD4+ T cells, *i.e.* HTLs, are purified from splenocytes of immunized animals and stimulated with each of the respective compositions (peptides encoded in the minigene). The HTL response is measured using a ^3H-thymidine incorporation proliferation assay, (*see, e.g.*, Alexander et al. Immunity 1:751-761, 1994). The results indicate the magnitude of the HTL response, thus demonstrating the *in vivo* immunogenicity of the minigene.

DNA minigenes, constructed as described in Example 11, may also be evaluated as a vaccine in combination with a boosting agent using a prime boost protocol. The boosting agent can consist of recombinant protein (*e.g.*, Barnett *et al.*, *Aids Res. and Human Retroviruses* 14, Supplement 3:S299-S309, 1998) or recombinant vaccinia, for example, expressing a minigene or DNA encoding the complete protein of interest (*see, e.g.*, Hanke *et al.*, *Vaccine* 16:439-445, 1998; Sedegah *et al.*, *Proc. Natl. Acad. Sci USA* 95:7648-53, 1998; Hanke and McMichael, *Immunol. Letters* 66:177-181, 1999; and Robinson *et al.*, *Nature Med.* 5:526-34, 1999).

For example, the efficacy of the DNA minigene used in a prime boost protocol is initially evaluated in transgenic mice. In this example, A2.1/K^b transgenic mice are immunized IM with 100 μg of a DNA minigene encoding the immunogenic peptides including at least one HLA-A2 supermotif-bearing

peptide. After an incubation period (ranging from 3-9 weeks), the mice are boosted IP with 10^7 pfu/mouse of a recombinant vaccinia virus expressing the same sequence encoded by the DNA minigene. Control mice are immunized with 100 μg of DNA or recombinant vaccinia without the minigene sequence, or with DNA encoding the minigene, but without the vaccinia boost. After an additional incubation period of two weeks, splenocytes from the mice are immediately assayed for peptide-specific activity in an ELISPOT assay. Additionally, splenocytes are stimulated *in vitro* with the A2-restricted peptide epitopes encoded in the minigene and recombinant vaccinia, then assayed for peptide-specific activity in an IFN-γ ELISA.

It is found that the minigene utilized in a prime-boost protocol elicits greater immune responses toward the HLA-A2 supermotif peptides than with DNA alone. Such an analysis can also be performed using HLA-A11 or HLA-B7 transgenic mouse models to assess CTL induction by HLA-A3 or HLA-B7 motif or supermotif epitopes.

The use of prime boost protocols in humans is described in Example 20.

Example 13. Peptide Composition for Prophylactic Uses

Vaccine compositions of the present invention are used to prevent cancer in persons who are at high risk for developing a tumor. For example, a polyepitopic peptide epitope composition (or a nucleic acid comprising the same) containing multiple CTL and HTL epitopes such as those selected in Examples 9 and/or 10, which are also selected to target greater than 80% of the population, is administered to an individual at high risk for prostate cancer. The composition is provided as a single polypeptide that encompasses multiple epitopes. The vaccine is administered in an aqueous carrier comprised of Freunds Incomplete Adjuvant. The dose of peptide for the initial immunization is from about 1 to about 50,000 μg, generally 100-5,000 μg, for a 70 kg patient. The initial administration of vaccine is followed by booster dosages at 4 weeks followed by evaluation of the magnitude of the immune response in the patient, by techniques that determine the presence of epitope-specific CTL populations in a PBMC sample. Additional booster doses are administered as required. The composition is found to be both safe and efficacious as a prophylaxis against cancer.

Alternatively, the polyepitopic peptide composition can be administered as a nucleic acid in accordance with methodologies known in the art and disclosed herein.

Example 14. Polyepitopic Vaccine Compositions Derived from Native TAA Sequences

A native TAA polyprotein sequence is screened, preferably using computer algorithms defined for each class I and/or class II supermotif or motif, to identify "relatively short" regions of the polyprotein that comprise multiple epitopes and is preferably less in length than an entire native antigen. This relatively short sequence that contains multiple distinct, even overlapping, epitopes is selected and used to generate a minigene construct. The construct is engineered to express the peptide, which corresponds to the native protein sequence. The "relatively short" peptide is generally less than 1000, 500, or 250 amino acids in length, often less than 100 amino acids in length, preferably less than 75 amino acids in length, and more preferably less than 50 amino acids in length. The protein sequence of the vaccine composition is selected because it has maximal number of epitopes contained within the sequence, *i.e.*, it has a high concentration of epitopes. As noted herein, epitope motifs may be nested or overlapping (*i.e.*,

frame shifted relative to one another). For example, with frame shifted overlapping epitopes, two 9-mer epitopes and one 10-mer epitope can be present in a 10 amino acid peptide. Such a vaccine composition is administered for therapeutic or prophylactic purposes.

The vaccine composition will preferably include, for example, three CTL epitopes and at least one HTL epitope from multiple prostate cancer-associated antigens. This polyepitopic native sequence is administered either as a peptide or as a nucleic acid sequence which encodes the peptide. Alternatively, an analog can be made of this native sequence, whereby one or more of the epitopes comprise substitutions that alter the cross-reactivity and/or binding affinity properties of the polyepitopic peptide.

The embodiment of this example provides for the possibility that an as yet undiscovered aspect of immune system processing will apply to the native nested sequence and thereby facilitate the production of therapeutic or prophylactic immune response-inducing vaccine compositions. Additionally such an embodiment provides for the possibility of motif-bearing epitopes for an HLA makeup that is presently unknown. Furthermore, this embodiment (absent analogs) directs the immune response to multiple peptide sequences that are actually present in native TAAs thus avoiding the need to evaluate any junctional epitopes. Lastly, the embodiment provides an economy of scale when producing nucleic acid vaccine compositions.

Related to this embodiment, computer programs can be derived in accordance with principles in the art, which identify in a target sequence, the greatest number of epitopes per sequence length.

Example 15. Polyepitopic Vaccine Compositions Comprising Epitopes From Multiple Tumor-Associated Antigens

The prostate cancer-associated antigen peptide epitopes of the present invention are used in combination with each other, or with peptide epitopes from other target tumor-associated antigens to create a vaccine composition that is useful for the treatment of prostate tumors from multiple patients. Furthermore, a vaccine composition comprising epitopes from multiple tumor antigens also reduces the potential for escape mutants due to loss of expression of an individual tumor antigen.

The composition can be provided as a single polypeptide that incorporates the multiple epitopes from the various TAAs, or can be administered as a composition comprising one or more discrete epitopes. Alternatively, the vaccine can be administered as a minigene construct or as dendritic cells which have been loaded with the peptide epitopes *in vitro*.

Example 16. Use of peptides to evaluate an immune response

Peptides of the invention may be used to analyze an immune response for the presence of specific CTL or HTL populations directed to a prostate cancer-associated antigen. Such an analysis may be performed using multimeric complexes as described, *e.g.*, by Ogg *et al.*, *Science* 279:2103-2106, 1998 and Greten *et al.*, *Proc. Natl. Acad. Sci. USA* 95:7568-7573, 1998. In the following example, peptides in accordance with the invention are used as a reagent for diagnostic or prognostic purposes, not as an immunogen.

In this example, highly sensitive human leukocyte antigen tetrameric complexes ("tetramers") are used for a cross-sectional analysis of, for example, tumor-associated antigen HLA-A*0201-specific CTL frequencies from HLA A*0201-positive individuals at different stages of disease or following immunization using a TAA peptide containing an A*0201 motif. Tetrameric complexes are synthesized as described (Musey *et al., N. Engl. J. Med.* 337:1267, 1997). Briefly, purified HLA heavy chain (A*0201 in this example) and β2-microglobulin are synthesized by means of a prokaryotic expression system. The heavy chain is modified by deletion of the transmembrane-cytosolic tail and COOH-terminal addition of a sequence containing a BirA enzymatic biotinylation site. The heavy chain, β2-microglobulin, and peptide are refolded by dilution. The 45-kD refolded product is isolated by fast protein liquid chromatography and then biotinylated by BirA in the presence of biotin (Sigma, St. Louis, Missouri), adenosine 5'triphosphate and magnesium. Streptavidin-phycoerythrin conjugate is added in a 1:4 molar ratio, and the tetrameric product is concentrated to 1 mg/ml. The resulting product is referred to as tetramer-phycoerythrin.

For the analysis of patient blood samples, approximately one million PBMCs are centrifuged at 300g for 5 minutes and resuspended in 50 µl of cold phosphate-buffered saline. Tri-color analysis is performed with the tetramer-phycoerythrin, along with anti-CD8-Tricolor, and anti-CD38. The PBMCs are incubated with tetramer and antibodies on ice for 30 to 60 min and then washed twice before formaldehyde fixation. Gates are applied to contain >99.98% of control samples. Controls for the tetramers include both A*0201-negative individuals and A*0201-positive uninfected donors. The percentage of cells stained with the tetramer is then determined by flow cytometry. The results indicate the number of cells in the PBMC sample that contain epitope-restricted CTLs, thereby readily indicating the extent of immune response to the TAA epitope, and thus the stage of tumor progression or exposure to a vaccine that elicits a protective or therapeutic response.

Example 17. Use of Peptide Epitopes to Evaluate Recall Responses

The peptide epitopes of the invention are used as reagents to evaluate T cell responses, such as acute or recall responses, in patients. Such an analysis may be performed on patients who are in remission, have a tumor, or who have been vaccinated with a prostate cancer-associated antigen vaccine.

For example, the class I restricted CTL response of persons who have been vaccinated may be analyzed. The vaccine may be any TAA vaccine. PBMC are collected from vaccinated individuals and HLA typed. Appropriate peptide epitopes of the invention that, optimally, bear supermotifs to provide cross-reactivity with multiple HLA supertype family members, are then used for analysis of samples derived from individuals who bear that HLA type.

PBMC from vaccinated individuals are separated on Ficoll-Histopaque density gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (GIBCO Laboratories), resuspended in RPMI-1640 (GIBCO Laboratories) supplemented with L-glutamine (2mM), penicillin (50U/ml), streptomycin (50 µg/ml), and Hepes (10mM) containing 10% heat-inactivated human AB serum (complete RPMI) and plated using microculture formats. A synthetic peptide comprising an epitope of the invention is added at 10 µg/ml to each well and HBV core 128-140 epitope is added at 1 µg/ml to each well as a source of T cell help during the first week of stimulation.

In the microculture format, 4 x 10^5 PBMC are stimulated with peptide in 8 replicate cultures in 96-well round bottom plate in 100 µl/well of complete RPMI. On days 3 and 10, 100 µl of complete RPMI and 20 U/ml final concentration of rIL-2 are added to each well. On day 7 the cultures are transferred into a 96-well flat-bottom plate and restimulated with peptide, rIL-2 and 10^5 irradiated (3,000 rad) autologous feeder cells. The cultures are tested for cytotoxic activity on day 14. A positive CTL response requires two or more of the eight replicate cultures to display greater than 10% specific ^{51}Cr release, based on comparison with uninfected control subjects as previously described (Rehermann, *et al., Nature Med.* 2:1104,1108, 1996; Rehermann *et al., J. Clin. Invest.* 97:1655-1665, 1996; and Rehermann *et al. J. Clin. Invest.* 98:1432-1440, 1996).

Target cell lines are autologous and allogeneic EBV-transformed B-LCL that are either purchased from the American Society for Histocompatibility and Immunogenetics (ASHI, Boston, MA) or established from the pool of patients as described (Guilhot, *et al. J. Virol. 66*:2670-2678, 1992).

Cytotoxicity assays are performed in the following manner. Target cells consist of either allogeneic HLA-matched or autologous EBV-transformed B lymphoblastoid cell line that are incubated overnight with the synthetic peptide epitope of the invention at 10 µM, and labeled with 100 µCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 hour after which they are washed four times with HBSS.

Cytolytic activity is determined in a standard 4 hour, split-well ^{51}Cr release assay using U-bottomed 96 well plates containing 3,000 targets/well. Stimulated PBMC are tested at effector/target (E/T) ratios of 20-50:1 on day 14. Percent cytotoxicity is determined from the formula: 100 x [(experimental release-spontaneous release)/maximum release-spontaneous release)]. Maximum release is determined by lysis of targets by detergent (2% Triton X-100; Sigma Chemical Co., St. Louis, MO). Spontaneous release is <25% of maximum release for all experiments.

The results of such an analysis indicate the extent to which HLA-restricted CTL populations have been stimulated by previous exposure to the TAA or TAA vaccine.

The class II restricted HTL responses may also be analyzed. Purified PBMC are cultured in a 96-well flat bottom plate at a density of 1.5x10^5 cells/well and are stimulated with 10 µg/ml synthetic peptide, whole antigen, or PHA. Cells are routinely plated in replicates of 4-6 wells for each condition. After seven days of culture, the medium is removed and replaced with fresh medium containing 10U/ml IL-2. Two days later, 1 µCi ^3H-thymidine is added to each well and incubation is continued for an additional 18 hours. Cellular DNA is then harvested on glass fiber mats and analyzed for ^3H-thymidine incorporation. Antigen-specific T cell proliferation is calculated as the ratio of ^3H-thymidine incorporation in the presence of antigen divided by the ^3H-thymidine incorporation in the absence of antigen.

Example 18. Induction Of Specific CTL Response In Humans

A human clinical trial for an immunogenic composition comprising CTL and HTL epitopes of the invention is set up as an IND Phase I, dose escalation study. Such a trial is designed, for example, as follows:

A total of about 27 male subjects are enrolled and divided into 3 groups:

Group I: 3 subjects are injected with placebo and 6 subjects are injected with 5 µg of peptide composition;

Group II: 3 subjects are injected with placebo and 6 subjects are injected with 50 μg peptide composition;

Group III: 3 subjects are injected with placebo and 6 subjects are injected with 500 μg of peptide composition.

After 4 weeks following the first injection, all subjects receive a booster inoculation at the same dosage. Additional booster inoculations can be administered on the same schedule.

The endpoints measured in this study relate to the safety and tolerability of the peptide composition as well as its immunogenicity. Cellular immune responses to the peptide composition are an index of the intrinsic activity of the peptide composition, and can therefore be viewed as a measure of biological efficacy. The following summarize the clinical and laboratory data that relate to safety and efficacy endpoints.

Safety: The incidence of adverse events is monitored in the placebo and drug treatment group and assessed in terms of degree and reversibility.

Evaluation of Vaccine Efficacy: For evaluation of vaccine efficacy, subjects are bled before and after injection. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

The vaccine is found to be both safe and efficacious.

Example 19. Therapeutic Use in Cancer Patients

Evaluation of vaccine compositions are performed to validate the efficacy of the CTL-HTL peptide compositions in cancer patients. The main objectives of the trials are to determine an effective dose and regimen for inducing CTLs in prostate cancer patients, to establish the safety of inducing a CTL and HTL response in these patients, and to see to what extent activation of CTLs improves the clinical picture of cancer patients, as manifested by a reduction in tumor cell numbers. Such a study is designed, for example, as follows:

The studies are performed in multiple centers. The trial design is an open-label, uncontrolled, dose escalation protocol wherein the peptide composition is administered as a single dose followed six weeks later by a single booster shot of the same dose. The dosages are 50, 500 and 5,000 micrograms per injection. Drug-associated adverse effects (severity and reversibility) are recorded.

There are three patient groupings. The first group is injected with 50 micrograms of the peptide composition and the second and third groups with 500 and 5,000 micrograms of peptide composition, respectively. The patients within each group are males, typically above the age of 50, and represent diverse ethnic backgrounds.

Example 20. Induction of CTL Responses Using a Prime Boost Protocol

A prime boost protocol similar in its underlying principle to that used to evaluate the efficacy of a DNA vaccine in transgenic mice, such as described in Example 12, can also be used for the administration of the vaccine to humans. Such a vaccine regimen can include an initial administration of,

for example, naked DNA followed by a boost using recombinant virus encoding the vaccine, or recombinant protein/polypeptide or a peptide mixture administered in an adjuvant.

For example, the initial immunization can be performed using an expression vector, such as one constructed in accordance with Example 11, in the form of naked nucleic acid administered IM (or SC or ID) in the amounts of 0.5-5 mg at multiple sites. The nucleic acid (0.1 to 1000 μg) can also be administered using a gene gun. Following an incubation period of 3-4 weeks, a booster dose is then administered. The booster can be recombinant fowlpox virus administered at a dose of 5-10^7 to 5x10^9 pfu. An alternative recombinant virus, such as an MVA, canarypox, adenovirus, or adeno-associated virus, can also be used for the booster, or the polyepitopic protein or a mixture of the peptides can be administered. For evaluation of vaccine efficacy, patient blood samples will be obtained before immunization as well as at intervals following administration of the initial vaccine and booster doses of the vaccine. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

Analysis of the results will indicate that a magnitude of response sufficient to achieve protective immunity against prostate cancer is generated.

Example 21. Administration of Vaccine Compositions Using Antigen Presenting Cells

Vaccines comprising peptide epitopes of the invention may be administered using antigen-presenting cells (APCs), or "professional" APCs such as dendritic cells (DC). In this example, the peptide-pulsed DC are administered to a patient to stimulate a CTL response *in vivo*. In this method, dendritic cells are isolated, expanded, and pulsed with a vaccine comprising peptide CTL and HTL epitopes of the invention. The dendritic cells are infused back into the patient to elicit CTL and HTL responses *in vivo*. The induced CTL and HTL then destroy (CTL) or facilitate destruction (HTL) of the specific target tumor cells that bear the proteins from which the epitopes in the vaccine are derived.

For example, a cocktail of epitope-bearing peptides is administered *ex vivo* to PBMC, or isolated DC therefrom, from the patient's blood. A pharmaceutical to facilitate harvesting of DC can be used, such as Progenipoietin™ (Monsanto, St. Louis, MO) or GM-CSF/IL-4. After pulsing the DC with peptides and prior to reinfusion into patients, the DC are washed to remove unbound peptides.

As appreciated clinically, and readily determined by one of skill based on clinical outcomes, the number of dendritic cells reinfused into the patient can vary (*see, e.g., Nature Med.* 4:328, 1998; *Nature Med.* 2:52, 1996 and *Prostate* 32:272, 1997). Although 2-50 x 10^6 dendritic cells per patient are typically administered, larger number of dendritic cells, such as 10^7 or 10^8 can also be provided. Such cell populations typically contain between 50-90% dendritic cells.

In some embodiments, peptide-loaded PBMC are injected into patients without purification of the DC. For example, PBMC containing DC generated after treatment with an agent such as Progenipoietin™ are injected into patients without purification of the DC. The total number of PBMC that are administered often ranges from 10^8 to 10^{10}. Generally, the cell doses injected into patients is based on the percentage of DC in the blood of each patient, as determined, for example, by immunofluorescence analysis with specific anti-DC antibodies. Thus, for example, if Progenipoietin™ mobilizes 2% DC in the

peripheral blood of a given patient, and that patient is to receive 5 x 10^6 DC, then the patient will be injected with a total of 2.5 x 10^8 peptide-loaded PBMC. The percent DC mobilized by an agent such as Progenipoietin™ is typically estimated to be between 2-10%, but can vary as appreciated by one of skill in the art.

The ability of DC to stimulate immune responses was evaluated in both *in vitro* and *in vivo* immune function assays. These assays include the stimulation of CTL hybridomas and CTL cell lines, and the *in vivo* activation of CTL.

DC Purification

Progenipoietin™-mobilized DC were purified from peripheral blood (PB) and spleens of Progenipoietin™-treated C57Bl/6 mice to evaluate their ability to present antigen and to elicit cellular immune responses. Briefly, DC were purified from total WBC and spleen using a positive selection strategy employing magnetic beads coated with a CD11c specific antibody (Miltenyi Biotec, Auburn CA). For comparison, *ex vivo* expanded DC were generated by culturing bone marrow cells from untreated C57Bl/6 mice with the standard cocktail of GM-CSF and IL-4 (R&D Systems, Minneapolis, MN) for a period of 7-8 days (Mayordomo *et al., Nature Med.* 1:1297-1302 (1995)). Recent studies have revealed that this *ex vivo* expanded DC population contains effective antigen presenting cells, with the capacity to stimulate anti-tumor immune responses (Celluzzi *et al., J. Exp. Med.* 83:283-287 (1996)).

The purities of Progenipoietin™-derived DC (100 µg/day, 10 days, SC) and GM-CSF/IL-4 *ex vivo* expanded DC were determined by flow cytometry. DC populations were defined as cells expressing both CD11c and MHC Class II molecules. Following purification of DC from magnetic CD11c microbeads, the percentage of double positive PB-derived DC, isolated from Progenipoietin™-treated mice, was enriched from approximately 4% to a range from 48-57% (average yield = 4.5 x 10^6 DC/animal). The percentage of purified splenic DC isolated from Progenipoietin™ treated mice was enriched from a range of 12-17% to a range of 67-77%. The purity of GM-CSF/IL-4 *ex vivo* expanded DC ranged from 31-41% (Wong *et al., J. Immunother.*, 21:32040 (1998)).

In Vitro Stimulation of CTL Hybridomas and CTL Cell Lines: Presentation of Specific CTL Epitopes

The ability of Progenipoietin™ generated DC to stimulate a CTL cell line was demonstrated *in vitro* using a viral-derived epitope and a corresponding epitope responsive CTL cell line. Transgenic mice expressing human HLA-A2.1 were treated with Progenipoietin™. Splenic DC isolated from these mice were pulsed with a peptide epitope derived from hepatitis B virus (HBV Pol 455) and then incubated with a CTL cell line that responds to the HBV Pol 455 epitope/HLA-A2.1 complex by producing IFNγ. The capacity of Progenipoietin™-derived splenic DC to present the HBV Pol 455 epitope was greater than that of two positive control populations: GM-CSF and IL-4 expanded DC cultures, or purified splenic B cells. A left shift in the response curve for Progenipoietin™-derived spleen cells versus the other antigen presenting cells revealed that these Progenipoietin™-derived cells required less epitope to stimulate maximal IFNγ release by the responder cell line.

The ability of *ex vivo* peptide-pulsed DC to stimulate CTL responses *in vivo* was also evaluated using the HLA-A2.1 transgenic mouse model. DC derived from Progenipoietin™-treated animals or control DC derived from bone marrow cells after expansion with GM-CSF and IL-4 were pulsed *ex vivo*

with the HBV Pol 455 CTL epitope, washed and injected (IV) into such mice. At seven days post immunization, spleens were removed and splenocytes containing DC and CTL were restimulated twice *in vitro* in the presence of the HBV Pol 455 peptide. The CTL activity of three independent cultures of restimulated spleen cell cultures was assessed by measuring the ability of the CTL to lyse ^{51}Cr-labeled target cells pulsed with or without peptide. Vigorous CTL responses were generated in animals immunized with the epitope-pulsed Progenipoietin™ derived DC as well as epitope-pulsed GM-CSF/IL-4 DC. In contrast, animals that were immunized with mock-pulsed Progenipoietin™-generated DC (no peptide) exhibited no evidence of CTL induction.

These data confirm that DC derived from Progenipoietin™ treated mice can be pulsed *ex vivo* with epitope and used to induce specific CTL responses *in vivo*. Thus, these data support the principle that Progenipoietin™-derived DC promote CTL responses in a model that manifests human MHC Class I molecules.

In vivo pharmacology studies in mice have demonstrated no apparent toxicity of reinfusion of pulsed autologous DC into animals.

Ex vivo activation of CTL/HTL responses

Alternatively, *ex vivo* CTL or HTL responses to a particular tumor-associated antigen can be induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptides. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cells, *i.e.*, tumor cells.

Example 22. Alternative Method of Identifying Motif-Bearing Peptides

Another way of identifying motif-bearing peptides is to elute them from cells bearing defined MHC molecules. For example, EBV transformed B cell lines used for tissue typing, have been extensively characterized to determine which HLA molecules they express. In certain cases these cells express only a single type of HLA molecule. These cells can then be infected with a pathogenic organism or transfected with nucleic acids that express the tumor antigen of interest. Thereafter, peptides produced by endogenous antigen processing of peptides produced consequent to infection (or as a result of transfection) will bind to HLA molecules within the cell and be transported and displayed on the cell surface.

The peptides are then eluted from the HLA molecules by exposure to mild acid conditions and their amino acid sequence determined, e.g., by mass spectral analysis (*e.g.*, Kubo *et al.*, *J. Immunol.* 152:3913, 1994). Because, as disclosed herein, the majority of peptides that bind a particular HLA molecule are motif-bearing, this is an alternative modality for obtaining the motif-bearing peptides correlated with the particular HLA molecule expressed on the cell.

Alternatively, cell lines that do not express any endogenous HLA molecules can be transfected with an expression construct encoding a single HLA allele. These cells may then be used as described, *i.e.*, they may be infected with a pathogenic organism or transfected with nucleic acid encoding an antigen of interest to isolate peptides corresponding to the pathogen or antigen of interest that have been

presented on the cell surface. Peptides obtained from such an analysis will bear motif(s) that correspond to binding to the single HLA allele that is expressed in the cell.

As appreciated by one in the art, one can perform a similar analysis on a cell bearing more than one HLA allele and subsequently determine peptides specific for each HLA allele expressed. Moreover, one of skill would also recognize that means other than infection or transfection, such as loading with a protein antigen, can be used to provide a source of antigen to the cell.

The above examples are provided to illustrate the invention but not to limit its scope. For example, the human terminology for the Major Histocompatibility Complex, namely HLA, is used throughout this document. It is to be appreciated that these principles can be extended to other species as well. Thus, other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent application cited herein are hereby incorporated by reference for all purposes.

TABLE I

SUPERMOTIFS	POSITION 2 (Primary Anchor)	POSITION 3 (Primary Anchor)	POSITION C Terminus (Primary Anchor)
A1	**T, I,** *L, V, M, S*		**F, W, Y**
A2	**L, I, V, M,** *A, T, Q*		**I, V,** *M, A, T, L*
A3	**V, S, M, A,** *T, L, I*		**R,K**
A24	**Y, F,** *W, I, V, L, M, T*		**F, I,** *Y, W, L, M*
B7	**P**		**V, I, L, F,** *M, W, Y, A*
B27	**R, H, K**		**F, Y, L,** *W, M, I, V, A*
B44	**E,** *D*		**F, W, L, I, M, V, A**
B58	**A, T, S**		**F, W, Y,** *L, I, V, M, A*
B62	**Q, L,** *I, V, M, P*		**F, W, Y,** *M, I, V, L, A*
MOTIFS			
A1	**T, S, M**		**Y**
A1		**D, E,** *A, S*	**Y**
A2.1	**L, M,** *V, Q, I, A, T*		**V,** *L, I, M, A, T*
A3	**L, M, V, I, S, A, T, F,** *C, G, D*		**K, Y, R,** *H, F, A*
A11	**V, T, M, L, I, S, A, G, N,** *C, D, F*		**K,** *R, Y, H*
A24	**Y, F, W,** *M*		**F, L, I, W**
A*3101	**M, V, T,** *A, L, I, S*		**R,** *K*
A*3301	**M, V, A, L, F,** *I, S, T*		**R, K**
A*6801	**A, V, T,** *M, S, L, I*		**R, K**
B*0702	**P**		**L, M, F,** *W, Y, A, I, V*
B*3501	**P**		**L, M, F, W, Y,** *I, V, A*
B51	**P**		**L, I, V, F,** *W, Y, A, M*
B*5301	**P**		**I, M, F, W, Y,** *A, L, V*
B*5401	**P**		**A, T, I, V,** *L, M, F, W, Y*

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE Ia

SUPERMOTIFS	POSITION	POSITION	POSITION
	2 (Primary Anchor)	3 (Primary Anchor)	C Terminus (Primary Anchor)
A1	**T, I,** *L, V, M, S*		**F, W, Y**
A2	**V,** *Q, A, T*		**I, V,** *L, M, A, T*
A3	**V, S, M, A,** *T, L, I*		**R, K**
A24	**Y, F,** *W, I, V, L, M, T*		**F, I,** *Y, W, L, M*
B7	**P**		**V, I, L, F,** *M, W, Y, A*
B27	**R, H, K**		**F, Y, L,** *W, M, I, V, A*
B58	**A, T, S**		**F, W, Y,** *L, I, V, M, A*
B62	**Q, L,** *I, V, M, P*		**F, W, Y,** *M, I, V, L, A*
MOTIFS			
A1	**T, S, M**		**Y**
A1		**D, E,** *A, S*	**Y**
A2.1	*V, Q, A, T**		**V,** *L, I, M, A, T*
A3.2	**L, M, V, I, S, A, T, F,** *C, G, D*		**K, Y, R,** *H, F, A*
A11	**V, T, M, L, I, S, A, G, N,** *C, D, F*		**K,** *R, H, Y*
A24	**Y ,F, W**		**F, L, I, W**

*If 2 is V, or Q, the C-term is not L

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE II

		POSITION 1	2	3	4	5	6	7	8	C-terminus
SUPERMOTIFS										
A1			1° Anchor T,I,*L,V,M,S*							1° Anchor F,W,Y
A2			1° Anchor L,I,V,M,*A, T,Q*							1° Anchor L,I,V,M,A,T
A3	preferred		1° Anchor V,S,M,A,*T, L,I*	Y,F,W, (4/5)			Y,F,W, (3/5)	Y,F,W, (4/5)	P, (4/5)	1°Anchor R,K
	deleterious	D,E (3/5); P, (5/5)		D,E, (4/5)						
A24			1° Anchor Y,F,*W,I,V, L,M,T*							1° Anchor F,I,*Y,W,L,M*
B7	preferred	F,W,Y (5/5) L,I,V,M, (3/5)	1°Anchor P	F,W,Y (4/5)					F,W,Y, (3/5)	1°Anchor V,I,L,F,*M,W,Y,A*
	deleterious	D,E (3/5); P(5/5); G(4/5); A(3/5); Q,N, (3/5)				D,E, (3/5)	G, (4/5)	Q,N, (4/5)	D,E, (4/5)	
B27			1° Anchor R,H,K							1° Anchor F,Y,L,*W,M,V,A*
B44			1° Anchor E,*D*							1° Anchor F,W,Y,L,I,M,V,A
B58			1° Anchor A,T,S							1° Anchor F,W,Y,*L,I,V,M,A*
B62			1° Anchor Q,L,*I,V,M, P*							1° Anchor F,W,Y,*M,I,V,L,A*

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus
MOTIFS										
A1 9-mer	preferred	G,F,Y,W,	1°Anchor S,T,M,	D,E,A,	Y,F,W,		P,	D,E,Q,N,	Y,F,W,	1°Anchor Y
	deleterious	D,E,		R,H,K,L,I,V M,P,	A,	G,	A,			
A1 9-mer	preferred	G,R,H,K	A,S,T,C,L,I V,M,	1°Anchor D,E,*A,S*	G,S,T,C,		A,S,T,C,	L,I,V,M,	D,E,	1°Anchor Y
	deleterious	A	R,H,K,D,E, P,Y,F,W,		D,E,	P,Q,N,	R,H,K,	P,G,	G,P,	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A1 10-mer	peferred	Y,F,W,	1°Anchor S,T,M	D,E,A,Q,N,	A,	Y,F,W,Q,N,		P,A,S,T,C,	G,D,E,	P,	1°Anchor Y
	deleterious	G,P,		R,H,K,G,L,I V,M,	D,E,	R,H,K,	Q,N,A	R,H,K,Y,F, W,	R,H,K,	A	
A1 10-mer	preferred	Y,F,W,	S,T,C,L,I,V M,	1°Anchor D,E,*A,S*	A,	Y,F,W,		P,G,	G,	Y,F,W,	1°Anchor Y
	deleterious	R,H,K,	R,H,K,D,E, P,Y,F,W,			P,	G,		P,R,H,K,	Q,N,	
A2.1 9-mer	preferred	Y,F,W,	1°Anchor L,M,*I,V,Q, A,T*	Y,F,W,	S,T,C,	Y,F,W,		A,	P	1°Anchor V,*L,I,M,A,T*	
	deleterious	D,E,P,		D,E,R,K,H			R,K,H	D,E,R,K,H			
A2.1 10-mer	preferred	A,Y,F,W,	1°Anchor L,M,*I,V,Q, A,T*	L,V,I,M,	G,		G,		F,Y,W,L, V,I,M,		1°Anchor V,*L,I,M,A,T*
	deleterious	D,E,P,		D,E,	R,K,H,A,	P,		R,K,H,	D,E,R,K, H,	R,K,H,	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A3	preferred	R,H,K,	1°Anchor L,M,V,I,S, A,T,F,*C,G D*	Y,F,W,	P,R,H,K,Y, F,W,	A,	Y,F,W,		P,	1°Anchor K,Y,R,*H,F,A*	
	deleterious	D,E,P,		D,E							
A11	preferred	A,	1°Anchor V,T,L,M,I, S,A,G,N,*C, D,F*	Y,F,W,	Y,FW,	A,	Y,F,W,	Y,FW,	P,	1°Anchor K,,*RY,H*	
	deleterious	D,E,P,						A	G,		
A24 9-mer	preferred	Y,F,W,R,H,K,	1°Anchor Y,F,W,*M*		S,T,C			Y,F,W,	Y,F,W,	1°Anchor F,L,I,W	
	deleterious	D,E,G,		D,E,	G,	Q,N,P,	D,E,R,H,K,	G,	A,Q,N,		
A24 10-mer	preferred		1°Anchor Y,F,W,*M*		P,	Y,F,W,P,		P,			1°Anchor F,L,I,W
	deleterious			G,D,E	Q,N	R,H,K	D,E	A	Q,N,	D,E,A,	
A3101	preferred	R,H,K,	1°Anchor M,V,T,*A,L, I,S*	Y,F,W,	P,		Y,F,W,	Y,F,W,	A,P,	1°Anchor R,*K*	
	deleterious	D,E,P,		D,E,		A,D,E,	D,E,	D,E,	D,E,		

		POSITION								
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A3301	preferred		1°Anchor M,V,A,L,F, *I,S,T*	Y,F,W				A,Y,F,W		1°Anchor R,K	
	deleterious	G,P		D,E							
A6801	preferred	Y,F,W,S,T,C,	1°Anchor A,V,T,*M,S, L,I*			Y,F,W,L,I, V,M		Y,F,W,	P,	1°Anchor R,K	
	deleterious	G,P,		D,E,G,		R,H,K,			A,		
B0702	preferred	R,H,K,F,W,Y,	1°Anchor P	R,H,K,		R,H,K,	R,H,K,	R,H,K,	P,A,	1°Anchor L,M,F,*W,Y,A, I,V*	
	deleterious	D,E,Q,N,P,		D,E,P,	D,E,	D,E,	G,D,E,	Q,N,	D,E,		
B3501	preferred	F,W,Y,L,I,V,M,	1°Anchor P	F,W,Y,				F,W,Y,		1°Anchor L,M,F,W,Y,*I, V,A*	
	deleterious	A,G,P,				G,	G,				

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
B51	preferred	L,I,V,M,F,W,Y,	1°Anchor P	F,W,Y,	S,T,C,	F,W,Y,		G,	F,W,Y,	1°Anchor L,I,V,F,*W, Y,A,M*	
	deleterious	A,G,P,D,E,R,H,K, S,T,C,				D,E,	G,	D,E,Q,N,	G,D,E,		
B5301	preferred	L,I,V,M,F,W,Y,	1°Anchor P	F,W,Y,	S,T,C,	F,W,Y,		L,I,V,M,F, W,Y,	F,W,Y,	1°Anchor I,M,F,W,Y, *A,L,V*	
	deleterious	A,G,P,Q,N,					G,	R,H,K,Q,N,	D,E,		
B5401	preferred	F,W,Y,	1°Anchor P	F,W,Y,L,I,V M,		L,I,V,M,		A,L,I,V,M,	F,W,Y,A,P,	1°Anchor A,T,I,V,*L, M,F,W,Y*	
	deleterious	G,P,Q,N,D,E,		G,D,E,S,T,C,		R,H,K,D,E,	D,E,	Q,N,D,G,E,	D,E,		

Italicized residues indicate less preferred or "tolerated" residues.
The information in Table II is specific for 9-mers unless otherwise specified.
Secondary anchor specificities are designated for each position independently.

Table III

		POSITION								
MOTIFS		1° anchor 1	2	3	4	5	1° anchor 6	7	8	9
DR4	preferred	F, M, Y, *L, I, V, W,*	M,	T,		I,	V, S, T, *C, P, A, L, I, M,*	M, H,		M, H
	deleterious				W,			R,		W, D, E
DR1	preferred	M, F, *L, I, V, W, Y,*			P, A, M, Q,		V, M, A, T, *S, P, L, I, C,*	M,		A, V, M
	deleterious		C	C, H	F, D	C, W, D		G, D, E,	D	
DR7	preferred	M, F, *L, I, V, W, Y,*	M,	W,	A,		I, V, M, S, A, *C, T, P, L,*	M,		I, V
	deleterious		C,		G,			G, R, D,	N	G
DR Supermotif		M, F, *L, I, V, W, Y,*					V, M, S, T, A, *C, P, L, I,*			

DR3 MOTIFS	1° anchor 1	2	3	1° anchor 4	5	1° anchor 6
motif a preferred	L, I, V, M, F, Y,			D		
motif b preferred	L, I, V, M, F, A, Y,			D, N, Q, E, S, T		K, R, H

Italicized residues indicate less preferred or "tolerated" residues. Secondary anchor specificities are designated for each position independently.

Table IV: HLA Class I Standard Peptide Binding Affinity.

ALLELE	STANDARD PEPTIDE	SEQUENCE (SEQ ID NO:)	STANDARD BINDING AFFINITY (nM)
A*0101	944.02	YLEPAIAKY	25
A*0201	941.01	FLPSDYFPSV	5.0
A*0202	941.01	FLPSDYFPSV	4.3
A*0203	941.01	FLPSDYFPSV	10
A*0205	941.01	FLPSDYFPSV	4.3
A*0206	941.01	FLPSDYFPSV	3.7
A*0207	941.01	FLPSDYFPSV	23
A*6802	1072.34	YVIKVSARV	8.0
A*0301	941.12	KVFPYALINK	11
A*1101	940.06	AVDLYHFLK	6.0
A*3101	941.12	KVFPYALINK	18
A*3301	1083.02	STLPETYVVRR	29
A*6801	941.12	KVFPYALINK	8.0
A*2402	979.02	AYIDNYNKF	12
B*0702	1075.23	APRTLVYLL	5.5
B*3501	1021.05	FPFKYAAAF	7.2
B51	1021.05	FPFKYAAAF	5.5
B*5301	1021.05	FPFKYAAAF	9.3
B*5401	1021.05	FPFKYAAAF	10

Table V. HLA Class II Standard Peptide Binding Affinity.

Allele	Nomenclature	Standard Peptide	Sequence (SEQ ID NO:)	Binding Affinity (nM)
DRB1*0101	DR1	515.01	PKYVKQNTLKLAT	5.0
DRB1*0301	DR3	829.02	YKTIAFDEEARR	300
DRB1*0401	DR4w4	515.01	PKYVKQNTLKLAT	45
DRB1*0404	DR4w14	717.01	YARFQSQTTLKQKT	50
DRB1*0405	DR4w15	717.01	YARFQSQTTLKQKT	38
DRB1*0701	DR7	553.01	QYIKANSKFIGITE	25
DRB1*0802	DR8w2	553.01	QYIKANSKFIGITE	49
DRB1*0803	DR8w3	553.01	QYIKANSKFIGITE	1600
DRB1*0901	DR9	553.01	QYIKANSKFIGITE	75
DRB1*1101	DR5w11	553.01	QYIKANSKFIGITE	20
DRB1*1201	DR5w12	1200.05	EALIHQLKINPYVLS	298
DRB1*1302	DR6w19	650.22	QYIKANAKFIGITE	3.5
DRB1*1501	DR2w2β1	507.02	GRTQDENPVVHFFKNIV TPRTPPP	9.1
DRB3*0101	DR52a	511	NGQIGNDPNRDIL	470
DRB4*0101	DRw53	717.01	YARFQSQTTLKQKT	58
DRB5*0101	DR2w2β2	553.01	QYIKANSKFIGITE	20

Table VI

HLA-supertype	Allelle-specific HLA-supertype members	
	Verified[a]	Predicted[b]
A1	A*0101, A*2501, A*2601, A*2602, A*3201	A*0102, A*2604, A*3601, A*4301, A*8001
A2	A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, A*6901	A*0208, A*0210, A*0211, A*0212, A*0213
A3	A*0301, A*1101, A*3101, A*3301, A*6801	A*0302, A*1102, A*2603, A*3302, A*3303, A*3401, A*3402, A*6601, A*6602, A*7401
A24	A*2301, A*2402, A*3001	A*2403, A*2404, A*3002, A*3003
B7	B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, B*7801	B*1511, B*4201, B*5901
B27	B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, B*7301	B*2701, B*2707, B*2708, B*3802, B*3903, B*3904, B*3905, B*4801, B*4802, B*1510, B*1518, B*1503
B44	B*1801, B*1802, B*3701, B*4402, B*4403, B*4404, B*4001, B*4002, B*4006	B*4101, B*4501, B*4701, B*4901, B*5001
B58	B*5701, B*5702, B*5801, B*5802, B*1516, B*1517	
B62	B*1501, B*1502, B*1513, B*5201	B*1301, B*1302, B*1504, B*1505, B*1506, B*1507, B*1515, B*1520, B*1521, B*1512, B*1514, B*1510

a. Verified alleles include alleles whose specificity has been determined by pool sequencing analysis, peptide binding assays, or by analysis of the sequences of CTL epitopes.

b. Predicted alleles are alleles whose specificity is predicted on the basis of B and F pocket structure to overlap with the supertype specificity.

Table VII
Prostate A01 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0101
PAP	122	11	
Kallikrein	147	11	
PSA	143	11	
Kallikrein	235	9	
PSA	231	9	0.0110
PSM	25	8	
PSM	25	9	
PAP	116	9	
PAP	311	9	0.7700
PAP	311	10	
PSM	531	11	
PSM	643	11	
PAP	12	9	
PSM	419	8	
PSM	13	8	
PSM	11	10	
PSM	393	10	
Kallikrein	241	9	
Kallikrein	66	9	
PSM	196	10	0.0160
PAP	347	10	
PSM	156	9	
PAP	201	10	
PSA	98	9	
PSM	630	10	
PSM	453	8	
PSM	106	8	
PAP	301	10	
PSM	137	8	
PSM	109	11	
PSM	586	10	
PAP	80	10	
PSM	64	10	
PAP	34	9	
PSM	480	9	
PAP	237	11	
PAP	240	8	
PSM	560	11	
PAP	358	11	
PAP	317	9	
PAP	317	10	
PSM	621	9	
PAP	168	10	
PSM	703	11	
PSM	716	10	
PAP	60	8	

Table VII
Prostate A01 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0101
PAP	216	11	
PAP	95	9	0.0980
PAP	170	8	
PSM	542	8	
PSM	542	11	
PSM	557	9	
PSM	557	10	0.0260
PSM	727	11	
PAP	18	8	
PSM	33	9	
PSM	33	10	
PSA	3	8	
Kallikrein	195	8	
PSA	191	8	
PSM	646	8	
PSM	546	11	
PSM	639	8	
PSM	529	9	0.0025
PAP	204	11	
PSM	104	10	0.4800
PAP	196	8	
PAP	196	11	
PSM	427	8	
PSM	680	8	
PAP	295	9	
PAP	74	11	
PSM	168	9	0.0001
PSM	311	9	
PSM	516	9	
PSM	516	10	
Kallikrein	158	8	
PSA	154	8	
PSM	403	8	
Kallikrein	149	9	
PSA	145	9	
PSM	224	11	
PSM	238	9	
Kallikrein	221	9	
PSA	217	9	
Kallikrein	52	8	
PSA	48	8	
PAP	128	11	
PSM	82	9	
PAP	270	11	
Kallikrein	94	8	0.0260
PSA	90	8	0.0260

Prostate A01 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0101
Kallikrein	34	10	
PSM	347	10	0.0048
PSM	130	10	
PSM	416	11	
PSM	373	9	
PSM	373	11	
PSA	69	9	
PSA	17	9	
PSM	226	9	
PSM	226	10	
PSM	512	10	
PSM	52	10	
PSM	200	10	
PSM	591	10	
PSM	157	8	
PSM	199	11	
PSM	514	8	
PSM	514	11	
PAP	193	11	
PSM	623	11	
PSM	718	8	
PSM	324	10	
Kallikrein	245	8	
PSA	241	8	
PSA	16	10	
Kallikrein	20	10	
PSM	34	8	
PSM	34	9	
PSA	70	8	
PSM	441	9	
Kallikrein	178	11	
PSM	668	8	
PAP	148	8	
PAP	148	11	
PAP	238	10	12.0000
PAP	194	10	
PAP	14	10	
PAP	14	11	
Kallikrein	179	10	
PSA	18	8	
PSM	117	11	
PAP	315	11	
PSM	268	10	0.0082
PAP	70	10	0.6200
PSM	561	10	
PAP	359	10	

Table VII
Prostate A01 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0101
PSM	26	8	
PSM	663	8	
PAP	114	11	
PSA	99	8	
PAP	117	8	
PSM	69	9	
PSM	51	11	
PSM	328	10	
PSM	153	9	
PAP	57	11	
PSM	678	9	
PSM	678	10	
PSA	15	11	
Kallikrein	19	11	
PAP	147	9	1.2000
PSM	267	11	
PAP	212	10	
PSM	550	10	
PAP	349	8	
PSM	290	10	
PSM	290	11	
PSA	236	10	0.0010
PAP	278	9	0.0031
PAP	54	10	
PSM	293	8	
Kallikrein	91	11	
PAP	276	11	
PSM	95	9	
PSM	218	11	
PSM	91	10	
PAP	72	8	
PSM	667	9	
PAP	69	11	
Kallikrein	22	8	
Kallikrein	39	9	
PSA	84	9	
PSA	182	10	
PSM	578	8	
PSA	87	11	
Kallikrein	72	10	
PSM	511	11	
PSM	527	11	
PAP	180	8	
PSM	440	10	
PSM	662	9	
PSM	400	11	

Table VII
Prostate A01 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0101
PAP	28	10	
PSM	414	8	
PSM	463	9	11.0000
Kallikrein	89	8	
PSM	129	11	
PSM	291	9	
PSM	291	10	
PSM	590	11	
PAP	130	9	
PSM	142	10	
PSM	631	9	
PAP	15	9	
PAP	15	10	
PAP	15	11	
PAP	13	8	
PAP	13	11	
PSA	237	9	0.0017
PSM	615	11	
PSM	695	11	
PSM	317	11	
PSM	348	9	0.0430
PAP	217	10	
PSA	67	11	
PAP	29	9	
PSM	626	8	
PSM	361	11	
PSM	461	11	
PSM	141	11	
Kallikrein	150	8	
PSA	146	8	
PSM	575	11	
PAP	145	11	
PSM	201	9	
PSM	372	10	
PSA	68	10	
PSM	225	10	
PSM	225	11	
PSM	690	11	
PSM	27	11	
PAP	30	8	
PSM	592	9	
Kallikrein	222	8	
PSA	218	8	
PSM	603	10	
PSM	660	11	
PSM	154	8	

Table VII
Prostate A01 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0101
PSM	154	11	
PAP	293	11	
Kallikrein	92	10	0.1500
PSA	88	10	0.1500
PAP	129	10	
Kallikrein	192	11	
PSA	188	11	
PSA	1	10	
PSM	394	9	
PSM	602	11	
Kallikrein	74	8	
PAP	206	9	0.0046
PSM	497	10	
PAP	84	9	
PAP	155	10	
PSM	228	8	
Kallikrein	188	8	
PSM	625	9	
PSM	537	10	
Kallikrein	243	10	
PSA	239	10	
PSM	371	11	
PSM	176	10	
PSM	176	11	

Table VIII

Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PSM	741	9	0.0002				
PSM	741	10					
PSM	742	8					
PSM	742	9					
PSM	735	8					
PSM	735	9					
PSM	735	11					
PSA	59	10	0.0002				
PSA	59	11	0.0010	0.0100	0.0140	0.0004	0.0018
Kallikrein	63	11	0.0003	0.0006	0.0450	0.0001	0.0004
PAP	121	9	0.0002				
PAP	121	11					
PSA	13	9	0.0002				
PSA	13	10	0.0002				
PAP	3	9					
PAP	3	10					
PAP	11	9	0.0002				
PAP	11	11					
PSM	392	8					
PAP	299	8					
PAP	299	9	0.0520				
PSM	711	9	0.0590	6.0000	7.2000	0.0250	0.0009
PAP	122	8					
PAP	122	10	0.0044				
Kallikrein	147	8	0.0230				
PSA	143	8	0.0230				
Kallikrein	235	8	0.0009	0.0200	0.0510	0.0001	-0.0001
Kallikrein	235	10	0.0003	0.0050	0.0028	0.0005	-0.0001
PSA	231	8	0.0002				
PSA	231	10	0.0008				
Kallikrein	9	9	0.0410	0.0038	0.1100	0.0066	-0.0001
Kallikrein	9	10	0.0180	0.2600	0.4000	0.0051	0.0012
PSM	25	10	0.0150				
PSM	25	11					
PAP	116	8					
PSM	302	8					
PSM	217	9					
PSM	217	10					
PSM	217	11					
PSA	181	8					
PSA	181	9	0.0002				
PSM	577	8					
PSM	577	11					
PSM	13	9	0.0002				
PSM	13	11					
PAP	227	9	0.0002				

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PAP	189	9	0.0005				
PSM	49	10					
PAP	274	10	0.0002				
PAP	274	11					
PSM	11	11					
PSA	44	8	0.0003				
PSM	365	8					
PSM	365	9	0.0001				
PSM	365	10	0.0002				
PSM	286	9	0.0042				
PSM	635	8					
PSM	635	9					
PSA	131	9	0.0001				
Kallikrein	17	9	0.0001	0.0026	0.0013	0.0020	0.0610
Kallikrein	17	10	0.0014	0.0510	0.0490	0.0035	0.0058
PSM	601	8					
PSM	601	11					
Kallikrein	41	8	-0.0001	0.0005	0.0011	0.0004	0.0003
PSM	22	8					
Kallikrein	198	11	0.0001	0.0003	0.0027	-0.0001	-0.0002
PSA	194	11	0.0013	0.0370	0.0250	0.0002	0.0081
Kallikrein	234	8	-0.0001	-0.0001	-0.0001	-0.0001	-0.0001
Kallikrein	234	9	0.0002	0.0013	0.1100	0.0004	0.0001
Kallikrein	234	11	0.0008	0.0033	0.0120	0.1700	-0.0002
PSA	230	9	0.0001				
PSA	230	11	0.0008	0.0130	0.0071	0.0016	0.0023
PSA	180	9	0.0002				
PSA	180	10	0.0001				
Kallikrein	184	9	-0.0001	0.0006	0.0025	0.0002	0.0012
Kallikrein	184	10	0.0074	0.0710	0.0200	0.0030	0.0071
PSA	62	8	0.0001				
PSA	62	9	0.0003				
PSA	62	10	0.0001				
Kallikrein	66	8	0.0001	0.0006	0.0006	-0.0001	-0.0001
Kallikrein	66	10	0.0001	0.0220	0.0083	0.0002	-0.0001
PAP	372	10	0.0002				
Kallikrein	14	8	0.0001	0.0001	0.0001	0.0012	0.0004
PSM	466	8					
PSM	466	9	0.0004				
PSA	169	11	0.0001				
Kallikrein	173	11	0.0002	0.0031	0.0020	0.0009	0.0007
PSM	422	8					
PSM	422	11					
PSM	710	10	0.0004				
PSM	301	9					
PSA	130	8	-0.0001	0.0003	-0.0001	-0.0001	0.0001

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PSA	130	10	0.0001				
PSM	714	11					
PSM	156	8					
PAP	201	9	0.0002				
PSA	171	9	0.0003				
PSA	171	11	0.0001				
Kallikrein	120	11	0.0022				
PSA	116	11	0.0022				
PSA	136	8	0.0001				
PSA	136	9	0.0003				
PSA	136	11	0.0041	0.0180	0.0100	0.0001	0.0009
Kallikrein	3	8	0.0001	-0.0002	-0.0001	-0.0001	0.0006
Kallikrein	3	10	0.0010	0.0180	0.0052	0.0230	0.0051
PSM	173	8					
PSM	173	10	0.0004				
Kallikrein	182	11	0.0001	0.0018	0.0130	0.0001	0.0170
PSM	191	10	0.0001				
PSM	191	11					
PSA	98	10	0.0001				
PSM	666	9					
PSM	666	11					
Kallikrein	207	11	0.0001	-0.0001	0.0005	-0.0001	0.0005
PAP	51	8					
Kallikrein	85	8	-0.0001	0.0001	-0.0001	-0.0001	0.0002
PSA	81	8	-0.0001	-0.0001	-0.0001	-0.0001	0.0016
PAP	230	9	0.0002				
PAP	290	9					
PAP	290	10					
PAP	290	11					
PSA	178	11	0.0001				
PAP	108	9					
PAP	108	10					
PAP	108	11					
PSM	114	10					
Kallikrein	134	8	-0.0001	-0.0001	-0.0001	-0.0001	0.0024
Kallikrein	134	10	0.0012	0.0230	0.0460	0.0004	0.0017
PAP	301	11					
PSM	48	11					
PSM	285	8					
PSM	285	10	0.0002				
PSM	641	10	0.0001				
PAP	266	9					
PAP	266	10					
PSM	397	8					
PSM	397	9	0.0002				
PSM	109	8					

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PSM	109	9	0.0028				
PSM	586	8					
PSM	64	11					
PAP	34	8					
PAP	237	8					
PAP	237	10	0.0008				
PAP	240	10	0.0002				
PSA	127	8	0.0001				
PSA	127	9	0.0001				
PSA	127	11	0.0001				
PSM	560	10	0.0001				
PAP	317	11					
PAP	328	8					
PAP	76	10					
PSM	87	10					
PAP	100	8					
PAP	100	10					
PSM	7	8					
PSM	7	9					
PSM	542	10	0.0002				
PAP	334	9	0.0002				
PAP	334	10					
PAP	334	11					
PSM	522	9	0.0002				
PSM	522	10					
PSM	727	8					
PSM	727	9					
PSM	727	10					
PSM	351	8					
PSM	351	9	0.0002				
PSM	351	11					
PAP	356	8					
PAP	356	9	0.0002				
PSM	418	11					
PAP	187	8					
PAP	187	11					
PSM	42	8					
PSM	42	9					
PSM	42	11					
PSM	61	10	0.0160				
PSM	670	10	0.0014				
PAP	18	9	0.0011				
PAP	20	11					
PSM	33	11					
PAP	92	11					
Kallikrein	165	10	0.0410	0.0940	1.1000	0.0068	0.0036

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PSA	3	9	0.0150				
PSA	3	11	0.0160				
PSA	161	10	0.0310				
PSM	73	8					
PSM	73	11					
Kallikrein	195	9	0.0220	0.0019	0.0160	0.0170	0.0006
PSA	191	9	0.0059				
PAP	164	8					
PAP	164	9					
PSM	525	11					
PSA	86	11					
PSM	333	10	0.0001				
PAP	221	8					
PAP	221	11					
PSM	77	8					
PSM	77	10					
PSM	737	9					
PSM	737	10	0.0001				
PAP	326	10					
PSA	12	10	0.0005				
PSA	12	11	0.1700	0.0220	0.0110	0.0006	0.0017
PSM	391	8					
PSM	391	9	0.0002				
PSM	24	11					
PSM	364	9	0.0001				
PSM	364	10	0.0002				
PSM	364	11					
Kallikrein	16	10	0.0017	0.0520	0.0380	0.0041	0.0057
Kallikrein	16	11	0.0001	0.0004	0.0004	0.0003	0.0003
PSM	282	8					
PSM	282	11					
PSM	529	10					
PSM	385	8					
PSM	385	9					
PSM	385	10	0.0002				
PSM	385	11					
PAP	248	11					
Kallikrein	225	11	0.0009	0.0014	0.0230	0.0001	0.0004
PSA	221	11	0.0001				
PAP	204	10	0.0002				
PSM	707	9	0.0210				
PSM	104	8					
PAP	196	10	0.0340				
PSM	427	9	0.0079				
PAP	305	11					
PSM	680	11					

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PSM	288	10	0.0340	1.6000	4.7000	0.0015	0.0260
Kallikrein	140	8	-0.0001	0.0003	-0.0001	-0.0001	-0.0001
Kallikrein	140	9	0.0002	0.0092	0.0013	0.0007	-0.0002
Kallikrein	140	11	0.0003	0.0200	0.0450	0.0006	0.0020
PAP	295	8					
Kallikrein	200	9	0.0002	0.0007	0.0015	-0.0001	-0.0002
PAP	74	8					
PSM	168	8					
PSM	168	10	0.0910	1.4000	1.4000	0.0230	0.0013
PSM	508	8					
PSM	582	10	0.0024				
PSM	582	11					
PAP	199	11					
PAP	68	8					
PSM	85	8					
PSM	85	9					
PSM	446	11					
PSM	224	9					
PSM	238	11					
Kallikrein	52	9	0.0003				
PSA	48	9	0.0003				
Kallikrein	52	10	0.0004				
PSA	48	10	0.0004				
Kallikrein	52	11	0.0002	0.0005	0.0005	0.0014	-0.0001
PSA	48	11	0.0002	0.0005	0.0005	0.0014	-0.0001
PAP	261	8					
PAP	261	11					
PSM	252	8					
PSM	252	10	0.0001				
PAP	128	8					
PAP	128	9	0.0034				
PAP	128	10	0.0016				
PSM	345	8					
PSM	345	9					
PSM	345	11					
PSM	82	11					
Kallikrein	177	9	0.0020	0.0049	0.0005	0.0009	0.0003
Kallikrein	177	11	0.0290	0.0520	0.1100	0.0088	0.0004
PSM	573	11					
PAP	270	8					
PAP	378	8					
PAP	144	10	0.0002				
PAP	144	11					
PSA	173	9	0.0001				
PSA	173	11	0.0024				
PSM	283	10	0.0001				

Table VIII

Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
Kallikrein	8	8	0.0001	-0.0002	-0.0001	-0.0001	0.0003
Kallikrein	8	10	0.0013	0.0500	0.0180	0.0180	0.0005
Kallikrein	8	11	0.0009	0.0032	0.0270	0.0100	0.0061
PSM	530	9					
PSM	642	9	0.0001				
PAP	188	10	0.0002				
PSM	130	9	0.0002				
PSM	416	8					
PSM	373	10	0.0003				
PSA	69	8	0.0010				
PAP	135	9	1.3000				
PAP	135	11					
PAP	267	8					
PAP	267	9	0.0001				
PAP	267	11					
PSM	258	11					
PSM	226	11					
PAP	284	8					
PAP	284	9	0.0019				
PAP	284	10	0.0610				
PSM	96	10					
Kallikrein	132	8	0.0001	0.0010	0.0001	-0.0001	0.0002
Kallikrein	132	10	0.0003	0.0084	0.0088	0.0004	0.0005
PSM	52	9					
PSM	52	11					
Kallikrein	226	10	0.0003	0.0100	0.0031	0.0005	0.0002
Kallikrein	226	11	0.0003	0.0150	0.0007	0.0013	0.0350
PSA	222	10	0.0003	0.0036	0.0030	0.0001	0.0003
PSA	222	11	0.0010	0.0120	0.0096	0.0001	0.0003
PSM	200	9	0.0001				
PSM	591	11					
PSM	659	10	0.0004				
PSM	659	11					
PSM	398	8					
PSM	66	9	0.0002				
PSM	59	9					
PSM	723	10	0.0001				
PSM	193	8					
PSM	193	9	0.0002				
PSM	193	10	0.0001				
PSM	193	11					
Kallikrein	131	8	0.0004	0.0002	0.0017	0.0002	-0.0001
Kallikrein	131	9	0.0047	0.0500	0.0420	0.0021	0.0002
Kallikrein	131	11	0.0002	0.0053	0.1700	0.0011	0.0006
PSM	199	10	0.0002				
PSM	187	8					

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PSM	514	10	0.0140				
PAP	282	10	0.0002				
PAP	282	11					
PSM	304	10	0.0003				
PSA	166	9	0.0190				
PSA	166	10	0.0370				
PAP	234	8					
PAP	234	10	0.0040				
PAP	234	11					
PAP	193	10	0.0026				
PSM	343	10	0.0042				
PSM	343	11					
PAP	251	8					
PSM	122	9	0.0002				
PSM	122	10	0.0001				
PSM	623	10	0.0002				
PSM	718	11					
PSM	207	8					
PSM	207	11					
PSM	341	9					
PSM	213	8					
PSM	213	10					
Kallikrein	137	11	0.0001	0.0004	0.0009	0.0012	0.0005
PSA	133	11	0.0014				
PSM	324	11					
Kallikrein	191	9	0.0035	0.0092	0.1900	0.1600	0.0004
Kallikrein	191	11	0.0010	0.0280	0.0280	0.0160	0.0036
PSA	187	9	0.0020				
Kallikrein	245	9	0.0001				
PSA	241	9	0.0001				
PAP	208	11					
PAP	120	10	0.0017				
PSM	219	8					
PSM	219	9	0.0002				
PSM	28	8					
PSM	28	11					
PSM	83	10	0.0001				
PSM	83	11					
PSM	110	8					
PAP	31	8					
PAP	31	9					
PAP	31	10	0.0002				
PAP	31	11					
PAP	8	9	0.0002				
PAP	283	9					
PAP	283	10					

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PAP	283	11					
PAP	7	8					
PAP	7	10	0.0061				
PSM	305	9	0.0001				
PAP	21	10	0.6000				
PAP	21	11					
PSM	34	10	0.0058				
PSM	428	8					
PSM	4	8					
PSM	4	9	0.0180				
PSM	4	10	0.0006				
PSM	4	11					
PAP	6	9	0.0120				
PAP	6	11					
PAP	306	10	0.0017				
PAP	306	11					
PSM	441	8					
PSM	441	10	0.0280	0.7500	1.5000	0.0043	0.0006
Kallikrein	123	8	0.0001				
PSA	119	8	0.0001				
PSA	119	10	0.0001				
PSA	119	11	0.0023	0.0140	0.0150	0.0002	0.0010
Kallikrein	123	10	0.0030	0.0290	0.9200	0.0010	0.0008
Kallikrein	123	11	0.0002	0.0007	0.0180	-0.0001	-0.0001
Kallikrein	178	8	0.0003	0.0073	0.0003	0.0021	-0.0001
Kallikrein	178	10	0.0030	0.0800	0.0280	0.0020	0.0042
PSM	116	8					
PAP	136	8					
PAP	136	10	0.0074				
PAP	136	11					
PSM	668	9	0.0110				
Kallikrein	121	10	0.0018				
PSA	117	10	0.0018				
PAP	113	8					
PAP	113	9	0.0071				
PAP	113	10	0.0037				
PAP	113	11					
PSM	469	9	0.0780	11.0000	4.8000	0.0340	0.0250
PSM	469	10	0.0046				
PSA	167	8					
PSA	167	9					
Kallikrein	171	8					
Kallikrein	171	9					
PSM	650	10					
PSM	650	11					
PSM	442	9					

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PSM	442	11					
PAP	258	10					
PAP	258	11					
PAP	296	11					
PSA	128	8	-0.0001	-0.0001	0.0002	-0.0001	0.0001
PSA	128	10	0.0002				
PSA	4	8	0.0003	-0.0001	0.0006	0.0007	0.0001
PSA	4	10	0.0018	0.0450	0.0820	0.0110	0.0910
PSA	4	11	0.0008	0.0014	0.0370	0.0025	0.0062
PSM	268	11					
PSA	162	9	0.0003				
PSA	162	11	0.0007	0.0087	0.0074	0.0004	0.0021
PSM	574	10					
PSM	574	11					
PSA	37	8	0.0001				
PSA	37	9	0.0003				
Kallikrein	217	10	0.0004				
PSA	213	10	0.0004				
Kallikrein	217	11	0.0007	0.0034	0.0033	0.0049	0.0041
PSA	213	11	0.0007	0.0034	0.0033	0.0049	0.0041
PSM	561	9					
PAP	40	11					
PSM	473	9	0.0001				
Kallikrein	54	8	0.0001				
PSA	50	8	0.0001				
Kallikrein	54	9	0.0001				
PSA	50	9	0.0001				
Kallikrein	54	10	0.0001				
PSA	50	10	0.0001				
Kallikrein	54	11	0.0001				
PSA	50	11	0.0001				
PSM	26	9	0.0280	0.0030	0.0004	0.1100	0.0003
PSM	26	10	0.0021				
Kallikrein	4	9	0.0020	0.0027	0.0085	0.0190	0.0002
PAP	263	9					
PSM	174	9					
PAP	298	9	0.0037				
PAP	298	10	0.0010				
Kallikrein	196	8	0.0014	0.0020	0.0018	0.0001	0.0002
PSA	192	8	0.0006	0.0012	0.0033	-0.0001	0.0001
Kallikrein	122	9	0.0610				
PSA	118	9	0.0610				
PSA	118	11	0.1400				
Kallikrein	122	11	0.0044	0.0072	0.2100	0.0019	0.0007
PAP	343	11					
PSM	663	9	0.4400	5.7000	5.8000	0.4900	0.0410

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PAP	232	10	0.0002				
PAP	373	9					
PSM	583	9	0.0170				
PSM	583	10	0.0140				
PSM	583	11					
PSM	451	11					
PSM	216	10	0.0002				
PSM	216	11					
PSM	69	10					
PSM	257	8					
PSM	51	8					
PSM	51	10					
PAP	119	11					
Kallikrein	79	8	0.0002	0.0035	0.0004	-0.0001	0.0004
PSM	3	9	0.0001				
PSM	3	10	0.0027				
PSM	3	11					
PSM	260	9	0.0007				
PSM	260	10	0.0002				
PSM	57	9	0.0026				
PSM	57	11					
Kallikrein	102	10	0.0043	0.0260	0.0400	0.0058	0.0020
PSM	357	9					
PSM	357	10	0.0001				
PSM	153	11					
PSM	231	9	0.0001				
PSA	125	8	-0.0001	-0.0001	-0.0001	-0.0001	-0.0001
PSA	125	10	0.0002				
PSA	125	11	0.0003	0.0028	0.0008	-0.0001	-0.0001
Kallikrein	129	8	0.0001	0.0003	-0.0001	-0.0001	-0.0001
Kallikrein	129	10	0.0011	0.0100	0.0320	0.0006	0.0002
Kallikrein	129	11	0.0002	0.0006	0.0017	-0.0001	0.0001
Kallikrein	146	9	0.0083	0.0210	0.0270	0.0002	0.0035
PSA	142	9	0.0083	0.0210	0.0270	0.0002	0.0035
PSM	273	11					
Kallikrein	240	8	0.0001	-0.0001	-0.0001	-0.0001	-0.0001
PAP	49	10	0.0002				
PSM	296	10	0.0001				
PSM	296	11					
PAP	134	8					
PAP	134	10	0.0075				
PAP	140	9	0.0002				
PSM	658	11					
PAP	352	8					
PAP	352	9	0.0001				
PSA	15	8	0.0001				

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
Kallikrein	19	8	0.0001	0.0002	-0.0001	-0.0001	-0.0001
PAP	5	8					
PAP	5	10	0.0004				
PSM	468	10	0.0008				
PSM	468	11					
PAP	147	8					
PAP	147	10	0.0006				
PSM	267	8					
Kallikrein	216	8	0.0001				
PSA	212	8	0.0001				
Kallikrein	216	11	0.0020				
PSA	212	11	0.0020				
PAP	212	11					
PSA	95	8	0.0002				
PSM	550	9	0.0002				
Kallikrein	99	8	0.0002	0.0008	0.0002	-0.0001	-0.0001
PSM	568	8					
PSM	568	9	0.0042				
PSM	568	10	0.0005				
PAP	365	9					
PAP	365	10					
PAP	365	11					
PSM	619	9					
PAP	64	8					
PAP	64	10					
PSM	166	9					
PSM	166	10					
PSA	185	8					
PSA	185	9					
PSA	185	11					
PSM	388	8					
PSM	388	11					
Kallikrein	57	8					
PSA	53	8					
PSA	53	11					
Kallikrein	57	11					
Kallikrein	142	9	0.0001				
PSA	138	9	0.0001				
Kallikrein	142	10	0.0084	0.0220	0.0520	0.0037	0.0005
PSA	138	10	0.0084	0.0220	0.0520	0.0037	0.0005
PSM	293	10					
PAP	362	9					
Kallikrein	91	10	0.0019	0.0099	0.0680	0.0022	0.0011
PSM	740	10	0.0006				
PSM	740	11					
PSM	79	8					

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PAP	276	8					
PAP	276	9	0.0002				
PAP	276	10					
PSM	95	11					
PSM	731	8					
PSM	731	9	0.0026				
PSM	731	11					
PSM	218	8					
PSM	218	9	0.0001				
PSM	218	10	0.0006				
PAP	72	10	0.0003				
PSM	667	8					
PSM	667	10	0.0510	0.1200	0.1100	0.0003	0.2700
PAP	297	10	0.0002				
PAP	297	11					
Kallikrein	39	10	0.0004	0.0097	0.0200	0.0005	0.0252
PSA	182	8	-0.0001	-0.0001	0.0001	-0.0001	-0.0001
PSA	182	11	0.0001				
PSA	35	10	0.0001				
PSA	35	11	0.0001				
PSM	578	10	0.0001				
PSM	578	11					
PSA	87	10	0.0001				
Kallikrein	72	9	0.0001	0.0021	0.0011	0.0025	0.0510
PAP	101	9	0.0002				
PAP	2	8					
PAP	2	10					
PAP	2	11					
PAP	10	10	0.0002				
PSM	673	9	0.0001				
PSM	534	10					
PAP	273	11					
PSA	43	8	-0.0001	-0.0001	0.0003	-0.0001	-0.0001
PSA	43	9	0.0002				
Kallikrein	186	8	-0.0001	-0.0001	0.0003	0.0001	-0.0001
Kallikrein	186	11	0.0007	0.0560	0.0016	0.0018	0.0009
PSM	354	8					
PSM	354	9	0.0004				
PSM	527	9	0.0001				
PAP	180	9	0.0006				
PAP	180	10	0.0048				
PAP	180	11					
PSM	440	8					
PSM	440	9	0.0001				
PSM	440	11					
PSM	649	11					

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PAP	257	8					
PAP	257	11					
PSA	121	8	0.0004				
PSA	121	9	0.0003				
PSA	121	11	0.0007				
Kallikrein	125	8	-0.0001	0.0005	0.0007	-0.0001	-0.0001
Kallikrein	125	9	-0.0001	-0.0002	0.0009	-0.0001	-0.0002
Kallikrein	125	11	0.0015	0.0043	0.0210	0.0002	0.0006
PSM	662	8					
PSM	662	10	0.5100	1.6000	1.3000	0.0930	0.0005
PSM	730	9					
PSM	730	10					
PSM	181	8					
PSM	414	10					
PAP	111	8					
PAP	111	10	0.0150				
PAP	111	11					
PSM	463	8					
PSM	463	11					
PSM	162	8					
PAP	287	10	0.0002				
PAP	115	8					
PAP	115	9	0.0043				
PSM	634	9	0.0001				
PSM	634	10					
Kallikrein	7	9	-0.0001	0.0006	0.0087	0.0006	0.0004
Kallikrein	7	11	0.0029	0.0066	0.0160	0.0100	0.0055
PSM	455	8					
PSM	455	10	0.0001				
Kallikrein	159	8	0.0001				
PSA	155	8	0.0001				
PSA	155	9	0.0001				
PSM	129	10	0.0001				
PSM	613	10					
PAP	130	8					
PSA	75	8	0.0003	0.0032	0.0028	-0.0001	-0.0001
PSA	75	11	0.0190				
PSM	631	10	0.0010				
PAP	15	8					
Kallikrein	175	9	0.0003	0.0720	0.0180	-0.0001	0.0004
Kallikrein	175	11	0.0390	1.9000	0.6900	0.0005	0.0004
PSM	322	8					
Kallikrein	104	8	0.0002	0.0007	0.0002	-0.0001	-0.0001
PSA	100	8	0.0020				
PAP	242	8					
Kallikrein	170	9	0.0100	0.0840	0.0240	0.0006	0.0031

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
Kallikrein	170	10	0.0099	0.4000	0.0920	0.0059	0.0008
PAP	13	9	0.0200				
PAP	13	10	0.0170				
PSM	472	10	0.0002				
PSM	615	8					
PSM	615	10	0.0001				
Kallikrein	35	8					
PSA	31	8					
PSA	31	9					
Kallikrein	71	10					
PSM	98	8					
PSM	98	11					
PSA	203	11	0.0005	0.0150	0.0092	0.0002	0.0035
PAP	106	8					
PAP	106	9					
PAP	106	11					
PSM	431	11					
PSM	348	8					
PSM	348	11					
PSM	338	9	0.0001				
PSM	107	9	0.0001				
PSM	107	10	0.0002				
PSM	107	11					
Kallikrein	11	8	0.0004	0.0006	0.0022	0.0003	-0.0001
Kallikrein	11	10	0.0024	0.0760	0.0065	0.0026	0.0035
Kallikrein	11	11	0.0100	0.0010	0.0007	0.0007	0.0005
PAP	217	11					
PSA	67	10	0.0001				
PAP	29	10	0.0031				
PAP	29	11					
PSM	626	10					
PSM	626	11					
PSA	7	8	0.0001				
PSA	7	10	0.0001				
PSA	7	11	0.0001				
PSM	554	8					
PSM	554	9	0.0073				
PSA	58	11	0.0005	0.0057	0.0085	0.0004	0.0105
PSM	14	8					
PSM	14	10					
PSM	415	9					
PAP	190	8					
PAP	171	11					
PAP	112	9	0.0650				
PAP	112	10	0.0065				
PAP	112	11					

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PAP	222	10	0.0002				
PAP	222	11					
PSM	461	9	0.0012				
PSM	461	10	0.0008				
PSA	5	9	0.0016				
PSA	5	10	0.0007				
PAP	231	8					
PAP	231	11					
Kallikrein	143	8					
PSA	139	8					
Kallikrein	143	9					
PSA	139	9					
PAP	335	8					
PAP	335	9					
PAP	335	10					
PSM	78	9					
PAP	275	9					
PAP	275	10					
PAP	275	11					
PSM	339	8					
PSM	339	11					
PAP	71	11					
Kallikrein	150	11	-0.0001	0.0009	0.0025	0.0005	0.1400
PSA	146	11	-0.0001	0.0009	0.0025	0.0005	0.1400
PAP	374	8					
PAP	291	8					
PAP	291	9					
PAP	291	10	0.0020				
PSM	575	9					
PSM	575	10	0.0005				
PAP	145	9	0.0002				
PAP	145	10	0.0001				
PSM	738	8					
PSM	738	9	0.0002				
PAP	292	8					
PAP	292	9	0.0044				
PAP	292	11					
PSM	734	8					
PSM	734	9					
PSM	734	10					
PSM	576	8					
PSM	576	9	0.0002				
PSA	38	8	-0.0001	-0.0001	-0.0001	-0.0001	-0.0001
PSM	12	10	0.0001				
Kallikrein	40	9	-0.0001	-0.0001	0.0002	0.0002	0.0004
PSM	447	10	0.0001				

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PSM	201	8					
PSM	358	8					
PSM	358	9	0.0002				
PSM	372	11					
PSA	68	9	0.0003				
PSM	225	8					
PAP	363	8					
PAP	363	11					
PSA	174	8	0.0001				
PSA	174	10	0.0008				
PSM	27	8					
PSM	27	9	0.1300	19.0000	0.3000	0.1200	0.0028
PAP	30	9	0.0590				
PAP	30	10	0.0021				
PAP	30	11					
Kallikrein	138	10	0.0008	0.0150	0.0110	0.0004	-0.0001
Kallikrein	138	11	-0.0001	0.0007	0.0003	0.0003	0.0006
PSM	115	9	0.0002				
PSM	592	10	0.0013				
PSM	592	11					
PSM	603	9	0.0002				
PSM	660	9	0.0001				
PSM	660	10	0.0003				
Kallikrein	5	8	0.0050	0.0790	0.0200	0.0024	0.0003
Kallikrein	5	11	0.0002	0.0011	0.0048	0.0004	0.0005
PSA	56	8	0.0001				
Kallikrein	60	8	0.0002	0.0034	0.0001	0.0001	0.0002
PSA	36	9	0.0001				
PSA	36	10	0.0003				
Kallikrein	53	8	0.0001				
PSA	49	8	0.0001				
Kallikrein	53	9	0.0200				
PSA	49	9	0.0200				
Kallikrein	53	10	0.0001				
PSA	49	10	0.0001				
Kallikrein	53	11	0.0130				
PSA	49	11	0.0130				
PAP	262	10	0.0008				
PSA	134	10	0.0001				
PSA	134	11	0.0021	0.0042	0.0014	0.0001	0.0003
PSM	739	8					
PSM	739	11					
PSM	253	9					
Kallikrein	192	8	-0.0001	0.0003	0.0005	0.0007	0.0007
Kallikrein	192	10	0.0008	0.0180	0.0068	0.0004	0.0030
PSA	188	8	0.0001	0.0002	0.0031	-0.0001	-0.0001

Table VIII
Prostate A02 Supermotif Peptides with Binding Information

Protein	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PSM	352	8					
PSM	352	10					
PSM	352	11					
PSA	8	9	0.0110				
PSA	8	10	0.0019				
PSA	8	11	0.0013	0.0005	0.0009	0.0011	0.0002
PSA	1	8	0.0002				
PSA	1	9	0.0008				
PSA	1	11	0.0069				
PSM	394	11					
Kallikrein	246	8	0.0001	0.0021	-0.0001	0.0001	-0.0001
PSA	242	8	0.0001	0.0021	-0.0001	0.0001	-0.0001
Kallikrein	246	11	0.0001	0.0001	0.0002	-0.0001	0.0004
PSA	242	11	0.0001	0.0001	0.0002	-0.0001	0.0004
Kallikrein	135	9	-0.0001	-0.0005	0.0007	0.0008	-0.0002
PSM	602	10	0.0001				
PSM	434	8					
PSM	434	9	0.0001				
Kallikrein	47	8	-0.0001	0.0003	0.0005	0.0001	0.0070
Kallikrein	47	9	-0.0001	0.0004	0.0067	0.0007	0.0310
PAP	226	8					
PAP	226	10	0.0002				
PSA	10	8	0.0005				
PSA	10	9	0.0005				
Kallikrein	252	8	0.0002	0.0120	0.1700	0.0002	-0.0001
PSA	248	8	0.0001				
PSM	20	8					
PSM	20	9	0.0180				
PSM	20	10	0.0120				
PAP	25	8					
PAP	25	11					
PAP	138	8					
PAP	138	9					
PAP	138	11					
Kallikrein	38	11					
PSA	34	11					
PSA	55	9	0.0008				
Kallikrein	59	9	0.0003	0.0018	0.0001	0.0160	0.0007
PSM	607	8					
PSM	607	10					
PSM	700	9	0.0013				
PSM	692	10					
PSM	179	10	0.0002				
PAP	310	9	0.0037				
Kallikrein	153	8	-0.0001	0.0009	0.0003	0.0003	0.0120
PSA	149	8	-0.0001	0.0009	0.0003	0.0003	0.0120

Table VIII

Prostate A02 Supermotif Peptides with Binding Information

Protein		Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
PSM	YAVVLRKYA	600	9					
PSM	YAYRRGIA	277	8					
PSM	YAYRRGIAEA	277	10					
PSM	YAYRRGIAEAV	277	11					
PSM	YINADSSI	449	8					
PAP	YIRKRYRKFL	84	10	0.0002				
PAP	YIRSTDVDRT	103	10					
PAP	YIRSTDVDRTL	103	11					
Kallikrein	YTKVVHYRKWI	243	11	0.0001	-0.0001	0.0004	-0.0001	0.0008
PSA	YTKVVHYRKWI	239	11	0.0001	-0.0001	0.0004	-0.0001	0.0008
PSM	YTLRVDCT	460	8					
PSM	YTLRVDCTPL	460	10	0.0015				
PSM	YTLRVDCTPLM	460	11					
PSM	YVAAFTVQA	733	9					
PSM	YVAAFTVQAA	733	10					
PSM	YVAAFTVQAAA	733	11					

Table IX

Prostate A03 Supermotif with Binding Data

Protein	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
PSA	59	8					
PSA	13	8					
PAP	3	8					
PSM	392	9					
PSM	711	8					
Kallikrein	235	11					
PSA	231	11					
PSM	531	9	0.0086	0.2700			
PAP	227	8	0.0003	0.0039			
PAP	227	10					
PSM	49	11					
PAP	274	8	0.0180	0.0700			
PAP	274	9	0.1000	1.2000			
PSM	11	9					
PSM	635	11					
Kallikrein	17	8					
PSM	393	8					
PSM	601	10	0.0026	0.0210			
Kallikrein	241	10					
Kallikrein	241	11					
Kallikrein	198	9					
PSA	194	9	0.0006	0.0015			
PSA	180	8					
PSA	180	11					
Kallikrein	184	8					
PSM	196	9					
PAP	347	9	0.0040	0.0006			
Kallikrein	14	11					
PSM	710	9	0.0006	0.0002			
PSM	301	8					
PSM	714	10	0.0003	0.0002			
PAP	201	8					
PSM	173	9					
Kallikrein	182	10					
PSM	191	9					
PSA	98	8	0.0003	0.0001			
PSA	98	11					
PSM	9	8					
PSM	9	9					
PSM	9	11					
PSM	630	8					
Kallikrein	116	10					
PSA	112	10					
PSM	453	11					
PSM	316	9	0.0032	0.0003			
PAP	51	9	0.0001	0.0001			

Table IX
Prostate A03 Supermotif with Binding Data

Protein	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
PSA	178	10	0.0007	0.0011			
PSM	114	9	0.0006	0.0010			
PSM	48	8					
PSM	641	9	0.0006	0.0002			
PAP	266	8					
PSM	397	10					
PSM	397	11					
PAP	166	8					
PAP	80	8					
PAP	80	9					
PAP	80	11					
PSM	64	8					
PSM	64	9					
PAP	34	10	0.0014	0.0037			
PSM	716	8					
PAP	95	11					
PSM	7	10					
PSM	7	11					
PAP	170	10	0.0004	0.0140			
PAP	170	11					
PSM	557	8					
PSM	675	10					
PSM	61	11					
PSM	37	8					
PAP	18	11					
PAP	20	9	0.0024	0.0004			
PSM	646	10	0.0003	0.0007			
PSM	506	9					
PSM	639	11					
PSM	333	9					
PSM	333	11					
PAP	37	11					
PSA	12	9	0.0150	0.0350			
PSM	391	10					
Kallikrein	16	9					
PSM	529	8					
PSM	529	11					
PAP	248	8					
PAP	248	10					
PSM	680	9	0.0460	0.0280			
PSM	311	10	0.0006	0.1400			
PSA	226	10					
Kallikrein	158	10					
PSM	430	11					
PSM	85	10					
PSM	403	9					

Table IX
Prostate A03 Supermotif with Binding Data

Protein	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
PSM	403	11					
PSM	360	11					
PSM	345	10					
Kallikrein	177	10					
PAP	314	9	0.2700	0.5300			
PSM	573	8					
PSM	347	8					
PSM	689	11					
PSM	202	9					
PSM	530	10					
PSM	642	8					
PSM	614	10	0.1900	0.1100			
PSM	52	8					
Kallikrein	25	9	0.0410	0.0190	0.0002	0.0006	0.001
PSA	21	9	0.0410	0.0190	0.0002	0.0006	0.001
PSM	200	8					
PSM	200	11					
PSM	591	8					
PSM	398	9	0.1700	0.0087			
PSM	398	10	0.0260	0.0006			
PSM	59	8					
PSM	723	8					
PSM	199	9	0.0740	1.0000			
PSM	610	8					
PAP	173	8					
PSM	491	9	0.4000	2.1000			
PSM	491	10	0.3200	0.0810			
PSM	655	8					
PSM	482	10	0.0044	0.0210			
PSA	66	8					
PSM	207	9	0.1600	0.1200			
PSM	213	11					
PSA	187	11					
Kallikrein	245	10	0.0450	0.0450			
PSA	241	10	0.0450	0.0450			
PSM	92	10	0.0031	0.0007			
PAP	21	8					
PSM	34	11					
Kallikrein	105	8					
PSA	101	8					
Kallikrein	123	9					
PAP	243	9	0.0760	0.2000			
PAP	243	11					
Kallikrein	178	9					
PAP	153	11					
Kallikrein	121	11					

Table IX
Prostate A03 Supermotif with Binding Data

Protein	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
PSM	469	11					
PAP	241	11					
PAP	244	8					
PAP	244	10	0.0520	0.0370			
Kallikrein	179	8					
PSA	57	8					
PSA	57	10	0.1400	0.0830			
Kallikrein	61	8					
Kallikrein	61	9					
PAP	315	8	0.0014	0.0100			
PSM	561	11					
PAP	40	8	0.0003	0.0002			
PSM	473	10					
PAP	263	10	0.0560	0.1200			
PAP	263	11					
PSM	174	8					
Kallikrein	196	11					
PSA	192	11					
Kallikrein	122	10					
PSM	663	11					
Kallikrein	103	10					
PSA	99	10	0.0070	0.0110			
PSM	216	8					
PSM	51	9					
Kallikrein	79	11					
PSM	247	9					
PSM	57	10					
Kallikrein	102	11					
PSM	589	10					
Kallikrein	70	8					
PSM	438	8					
PSM	231	10					
PSA	125	9	0.0002	0.0002	0.0004	0.0006	0.0001
Kallikrein	129	9					
PSM	273	8					
PSM	273	9	0.0001	0.0002			
Kallikrein	240	11					
PAP	49	11					
PSM	296	9					
PSM	678	11					
PSA	95	9	0.2400	0.0370	0.0002	0.0006	0.0001
PSA	95	11					
Kallikrein	99	9					
PSM	721	9					
PSM	721	10	0.0003	0.0002			
PSA	236	11					

Table IX
Prostate A03 Supermotif with Binding Data

Protein	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
PSM	502	10					
PAP	224	11					
PSM	91	11					
PAP	152	8					
PSA	182	9	0.0060	0.0140	0.0028	0.0014	0.0051
PSA	35	9	0.0021	0.0018			
PAP	101	11					
PAP	2	9	0.1500	0.1200			
PAP	273	9	0.0210	0.0600			
PAP	273	10	0.0053	0.0250			
Kallikrein	24	10	0.0460	0.0670			
PSA	20	10	0.0460	0.0670			
PSM	354	10	0.3700	0.4300			
PSM	527	8					
PSM	527	10					
PSM	400	8					
PAP	28	9	0.0490	0.1100			
PSM	181	10					
PSM	312	9	0.0006	0.0012			
PSM	10	8					
PSM	10	10					
PSM	455	9					
Kallikrein	159	9					
Kallikrein	159	11					
PSA	155	11					
PSM	613	11					
PSM	590	9	0.0006	0.0220			
Kallikrein	104	9					
PSA	100	9	0.0024	0.0470			
PAP	242	10	0.4900	2.3000			
PSM	472	8					
PSM	472	11					
PSM	492	8					
PSM	492	9	1.0000	2.0000			
PAP	245	9	1.1000	0.8000			
PAP	245	11					
PSA	237	10	0.2800	0.2300			
PSA	237	11					
PSM	615	9	0.1100	0.0720			
Kallikrein	117	9	0.0039	1.2000			
PSA	113	9	0.0039	1.2000			
PSM	454	10	0.0007	0.0910			
PSM	45	11					
PSM	317	8					
PSM	431	10	0.0005	0.0016			
PAP	29	8	0.0017	0.0061			

Table IX

Prostate A03 Supermotif with Binding Data

Protein	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
PSM	554	11					
PSA	58	9	0.0094	0.0140			
Kallikrein	62	8					
PSM	404	8					
PSM	404	10	0.0007	0.0002			
PSM	404	11					
PAP	171	9	0.0006	0.0078			
PAP	171	10	0.0007	0.0001			
PSM	361	10	0.0003	0.0002			
PAP	39	9	0.0006	0.0002			
PSM	12	8					
PSM	201	10					
PSM	690	10	0.5400	0.7900			
PSM	115	8					
PSM	603	8					
PSA	56	9	0.0002	0.0005			
PSA	56	11					
Kallikrein	60	9					
Kallikrein	60	10					
PSA	36	8					
PAP	262	11					
PSM	627	11					
PSA	188	10	0.0003	0.0120			
PAP	38	10					
Kallikrein	246	9	0.0072	0.0930	0.5500	0.0490	0.0028
PSA	242	9	0.0072	0.0930	0.5500	0.0490	0.0028
PSM	602	9	0.0390	0.0660			
PAP	226	9	0.0006	0.0002			
PAP	226	11					
PSA	10	11					
PAP	25	9	0.0035	0.0150			
PSA	55	10	0.0004	0.0001			
Kallikrein	59	10					
Kallikrein	59	11					
PSM	607	11					
PSM	692	8					
PSM	179	9					
PSM	600	11					
PAP	84	8					
PAP	103	9					
PAP	155	9					
PSM	471	9	0.0600	0.5400			
PSM	537	9					
Kallikrein	243	8					
PSA	239	8					
Kallikrein	243	9	0.0006	0.0580	1.2000	2.8000	1.3000

Table IX
Prostate A03 Supermotif with Binding Data

Protein	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
PSA	239	9	0.0006	0.0580	1.2000	2.8000	1.3000
PSM	371	8					

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	674	8	
PSM	60	11	
PSM	736	11	
PAP	299	8	
PAP	299	9	
PAP	122	10	
PAP	122	11	
Kallikrein	147	11	
PSA	143	11	
Kallikrein	235	9	
PSA	231	8	
PSA	231	9	
PSM	25	8	
PSM	25	9	
PSM	25	10	
PSM	25	11	
PAP	116	8	
PAP	116	9	0.0150
PSM	13	8	
PSM	13	9	
PAP	227	9	
PAP	189	9	
PSM	49	10	
PAP	274	10	
PAP	274	11	
PSM	11	10	
PSM	11	11	
PSM	365	9	
PSM	365	10	
PSM	635	8	
Kallikrein	17	9	
PSM	393	10	
PSM	601	11	
Kallikrein	241	9	
PSM	724	9	
PSM	724	10	
PSM	448	9	0.0190
Kallikrein	187	9	
Kallikrein	187	10	
Kallikrein	187	11	
PSA	62	8	
PSA	62	9	
PSA	62	10	
Kallikrein	66	9	
Kallikrein	66	10	
Kallikrein	14	8	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	466	8	
Kallikrein	173	11	
Kallikrein	152	9	0.1700
PSA	148	9	0.1700
PSM	652	8	
PSM	652	10	
PSM	520	9	
PSM	520	11	
PSM	184	9	
PSM	184	11	
PAP	186	9	0.0002
PSM	156	9	
PAP	201	10	
PSA	136	9	
Kallikrein	3	8	
PSM	191	10	
PSA	98	9	0.0001
PSA	98	10	
Kallikrein	207	11	
PAP	51	8	
PAP	230	9	
PAP	290	9	
PAP	290	11	
PAP	108	10	
Kallikrein	134	8	
PAP	301	10	
PSM	599	9	
PSM	233	10	
PSM	102	9	
PSM	425	10	
Kallikrein	164	8	
PSA	160	8	
Kallikrein	194	8	
Kallikrein	194	9	
PAP	176	9	
PSM	505	8	
PSM	505	11	
PSM	641	10	
PSM	137	8	
PSM	397	9	
PSM	109	8	
PSM	109	9	
PSM	109	11	
PSM	586	8	
PSM	586	10	
PAP	80	10	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	64	10	
PSM	64	11	
PAP	34	9	
PSM	480	9	
PAP	237	8	
PAP	237	10	
PAP	237	11	
PAP	240	8	
PAP	240	10	
PSA	127	9	
PSA	127	11	
PSM	560	10	
PSM	560	11	
PAP	358	11	
PAP	317	9	
PAP	317	10	
PSM	621	9	0.0010
PAP	170	8	
PSM	542	8	
PSM	542	10	
PSM	542	11	
PAP	334	9	
PAP	334	10	
PAP	334	11	
PSM	557	9	
PSM	557	10	
PSM	522	9	
PSM	727	11	
PSM	351	9	
PSM	433	9	
PSM	433	10	
PSM	276	8	
PAP	324	8	
PAP	83	10	0.0067
PAP	83	11	
PSM	185	8	
PSM	185	10	
PSM	32	8	
PSM	32	10	0.0026
PSM	32	11	
PAP	23	9	0.0017
PAP	187	8	
PAP	187	11	
PSM	42	11	
PSM	61	10	
PSM	670	10	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PAP	18	8	
PAP	18	9	
PSM	33	9	
PSM	33	10	
PSM	33	11	
PSA	3	8	
PSA	3	9	
PSM	73	8	
PSM	73	11	
Kallikrein	195	8	
PSA	191	8	
PSM	639	8	
PSM	737	10	
PAP	24	8	
PSM	565	8	
PSM	565	10	1.1000
PSM	487	8	
PSM	487	11	
PSM	31	8	
PSM	31	9	0.0190
PSM	31	11	
PAP	66	8	
PAP	66	10	
PSM	36	8	
PAP	17	8	
PAP	17	9	0.0016
PAP	17	10	0.0007
PSM	282	8	
PSM	282	11	
PSM	529	9	
PAP	248	11	
PAP	204	10	
PAP	204	11	
PSM	707	9	
PSM	104	10	
PAP	196	8	
PAP	196	10	
PAP	196	11	
PSM	427	8	
PAP	305	11	
PSM	680	8	
PSM	288	10	
Kallikrein	140	9	
PAP	295	9	
PAP	74	8	
PAP	74	11	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	168	9	
PSM	508	8	
PSM	582	10	0.0002
PSM	85	8	
PSM	403	8	
Kallikrein	149	9	
PSA	145	9	
PSM	446	11	
PSM	224	11	
PSM	238	9	
PSM	238	11	
Kallikrein	221	9	
PSA	217	9	
Kallikrein	52	8	
PSA	48	8	
Kallikrein	52	10	
PSA	48	10	
PAP	261	8	
PAP	261	11	
PSM	252	8	
PSM	252	10	
PAP	128	8	
PAP	128	9	
PAP	128	10	
PAP	128	11	
Kallikrein	46	9	
Kallikrein	28	11	
PSA	24	11	
Kallikrein	156	10	0.0001
PSA	152	10	0.0001
Kallikrein	156	11	
PSA	152	11	
PSM	409	8	
PSM	409	9	
PSM	409	10	0.0540
PSM	150	8	
PSM	271	9	
PSM	548	9	
PSM	298	8	
PSM	298	9	
PSM	345	11	
PSM	82	9	
PSM	82	11	
PSM	573	11	
PAP	270	8	
PAP	270	11	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PAP	144	10	
PAP	144	11	
PSM	112	8	
PAP	78	8	
Kallikrein	248	10	0.0550
PSA	244	10	0.0550
PSM	130	9	
PSM	130	10	
PSM	416	11	
PSM	373	9	
PSM	373	11	
PSA	69	8	
PSA	69	9	
PAP	267	11	
PSM	258	11	
PSA	17	9	
PSM	226	9	
PSM	226	10	
Kallikrein	132	8	
Kallikrein	132	10	
PSM	52	10	
PSM	52	11	
Kallikrein	226	11	
PSA	222	11	
PSM	200	10	
PSM	591	10	
PSM	659	10	
PSM	659	11	
PSM	157	8	
PSM	398	8	
PAP	131	8	
PAP	131	11	
PAP	205	9	0.0024
PAP	205	10	
PSM	691	10	
PSM	708	8	
PSM	355	8	
PSM	72	9	
PSA	190	9	0.0310
PSM	645	9	
PSM	545	8	
PSM	564	9	
PSM	564	11	
PSM	193	9	
PSM	193	10	
Kallikrein	131	9	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
Kallikrein	131	11	
PSM	199	11	
PSM	187	8	
PSM	514	8	
PSM	514	11	
PSA	166	10	
PAP	234	8	
PAP	234	10	
PAP	234	11	
PAP	193	10	
PAP	193	11	
PSM	122	9	
PSM	122	10	
PSM	623	10	
PSM	623	11	
PSM	718	8	
PSM	324	10	
Kallikrein	191	11	
Kallikrein	245	8	
PSA	241	8	
Kallikrein	245	9	
PSA	241	9	
PSM	606	9	12.0000
PSM	606	11	
PSM	699	10	
PSM	699	11	
PSM	417	10	
PSM	143	9	
PAP	22	10	0.0045
PAP	202	9	
PSA	76	11	
PAP	19	8	
PAP	123	9	0.0033
PAP	123	10	0.0140
PSM	632	8	
PSM	632	11	
PSA	16	10	
Kallikrein	20	10	
PAP	7	8	
PAP	7	10	
PAP	21	11	
PSM	34	8	
PSM	34	9	
PSM	34	10	
PSA	70	8	
PAP	6	9	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PAP	6	11	
PAP	306	10	
PSM	441	9	
PSM	441	10	
PSA	119	10	
Kallikrein	123	10	
Kallikrein	178	11	
PSM	668	8	
PSM	668	9	0.0075
PAP	113	8	
PAP	113	11	
PSM	469	9	
PSA	128	8	
PSA	128	10	
PAP	315	11	
PSA	4	8	
PSM	268	10	
PSA	162	11	
PAP	70	10	0.0022
PSM	574	10	
Kallikrein	217	10	
PSA	213	10	
PSM	561	9	
PSM	561	10	
PAP	40	11	
PAP	359	10	
PSM	473	9	
Kallikrein	54	8	
PSA	50	8	
PSM	26	8	
PSM	26	9	
PSM	26	10	
PAP	263	9	
PAP	213	9	0.4400
PAP	213	11	
PSA	96	11	0.1200
PAP	318	8	
PAP	318	9	2.5000
PSM	551	9	
PSM	551	11	
PAP	154	11	
PSM	74	10	0.2300
PSM	227	8	
PSM	227	9	0.4400
PSA	238	8	
PSA	238	11	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	669	8	
PSM	669	11	
PSA	118	11	
Kallikrein	122	11	
PAP	343	11	
PSM	663	8	
PSM	663	9	
PAP	232	10	
PAP	117	8	
PSM	583	9	
PSM	583	11	
Kallikrein	1	8	
Kallikrein	1	10	
PSM	470	8	
PSM	89	8	
PSM	336	9	
PSM	336	11	
PSM	638	9	0.0001
PSM	76	8	
PSM	69	9	
PSM	51	8	
PSM	51	11	
PSM	260	9	
PSM	57	9	
Kallikrein	102	10	
PSM	328	10	
PSM	153	9	
PSM	540	10	
PSM	178	8	
PSM	178	9	0.7700
PSM	178	11	
PSM	459	11	
PSM	594	11	
PAP	157	8	
PAP	157	11	
PSM	160	10	
PSM	685	8	
PAP	49	10	
PSM	296	10	
PSM	296	11	
PAP	57	11	
PAP	134	8	
PAP	140	9	
PSM	658	11	
PAP	352	8	
PSM	678	9	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	678	10	
PSA	15	11	
Kallikrein	19	11	
PAP	5	10	
PSM	468	10	
PAP	147	8	
PAP	147	9	
PAP	147	10	
PSM	267	11	
Kallikrein	216	8	
PSA	212	8	
Kallikrein	216	11	
PSA	212	11	
PAP	212	10	
PSA	95	8	
PSM	550	10	
Kallikrein	99	8	
PAP	54	10	
PSM	293	8	
Kallikrein	91	10	
Kallikrein	91	11	
Kallikrein	37	11	
PAP	309	10	0.0240
PAP	309	11	
PAP	183	9	0.1100
PSM	326	8	
PAP	276	8	
PAP	276	9	
PAP	276	10	
PAP	276	11	
PSM	95	9	
PSM	95	11	
PSM	218	9	
PSM	218	10	
PSM	218	11	
PSM	91	10	
PAP	72	8	
PAP	72	10	
PSM	667	9	
PSM	667	10	
PAP	69	11	
PAP	297	10	0.0001
PAP	297	11	
Kallikrein	39	9	
PSA	84	9	
PSA	182	10	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSA	182	11	
PSM	578	8	
PSM	578	10	
PSA	87	10	
PSA	87	11	
Kallikrein	72	9	
Kallikrein	72	10	
PSA	54	10	0.0007
Kallikrein	58	10	
PAP	355	10	0.0037
PAP	163	10	0.0001
PSM	511	11	
PSM	354	9	
PSM	527	11	
PAP	180	8	
PAP	180	9	
PSM	440	10	
PSM	440	11	
PSM	649	11	
PAP	257	11	
PSA	121	8	
Kallikrein	125	8	
PSM	662	8	
PSM	662	9	
PSM	662	10	
PSM	181	8	
PSM	414	8	
PAP	111	10	
PSM	463	8	
PSM	463	9	
PSM	463	11	
Kallikrein	89	8	
PSM	19	8	
PSM	19	10	
PAP	88	10	0.0057
PSM	536	11	
PSM	401	10	
PSM	704	9	
PSM	704	10	
PSA	91	9	0.0007
PSA	91	11	
Kallikrein	95	9	
Kallikrein	95	11	
PSM	455	8	
Kallikrein	159	8	
PSA	155	8	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	129	10	
PSM	129	11	
PSM	291	9	
PSM	291	10	
PSM	613	10	
PSM	590	11	
PAP	130	8	
PAP	130	9	
PSM	142	10	
PSM	631	9	
PAP	15	8	
PAP	15	9	
PAP	15	10	
PAP	15	11	
Kallikrein	175	9	
Kallikrein	104	8	
PSA	100	8	
PAP	242	8	
Kallikrein	170	9	
Kallikrein	170	10	
PAP	13	8	
PAP	13	9	
PAP	13	10	
PAP	13	11	
PSM	472	10	
PSA	237	9	
PSM	615	8	
PSM	615	11	
PSA	203	11	
PAP	106	8	
PAP	106	9	
PSM	431	11	
PSM	348	8	
PSM	348	9	
PSM	338	9	
PSM	107	10	
PSM	107	11	
Kallikrein	11	10	
Kallikrein	11	11	
PAP	217	10	
PSA	67	10	
PSA	67	11	
PAP	29	9	
PSM	626	8	
PSA	7	10	
PSA	7	11	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	554	8	
PAP	225	8	
PAP	225	11	
PSM	420	9	
PSM	420	10	
Kallikrein	228	9	
PSA	224	9	0.0001
PAP	62	9	0.0013
PSM	318	10	
PSM	496	11	
PAP	96	8	
PAP	96	9	0.2600
PAP	279	8	
PSM	241	8	
PSM	118	10	
PSM	118	11	
PAP	190	8	
PAP	171	11	
PAP	112	9	
PAP	222	11	
PSM	361	11	
PSM	461	9	
PSM	461	10	
PSM	461	11	
PAP	231	8	
PAP	231	11	
Kallikrein	150	8	
PSA	146	8	
Kallikrein	150	11	
PSA	146	11	
PAP	291	8	
PAP	291	10	
PSM	575	9	
PSM	575	11	
PAP	145	9	
PAP	145	10	
PAP	145	11	
PSM	738	9	
PAP	292	9	
PSA	9	8	
PSA	9	9	0.1100
PSA	9	10	0.3600
PSM	558	8	
PSM	558	9	
PSM	624	9	
PSM	624	10	3.2000

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	584	8	
PSM	584	10	
PSM	523	8	
PSA	2	9	2.1000
PSA	2	10	0.0062
PSA	85	8	
PAP	41	10	0.0005
PSM	201	9	
PSM	372	10	
PSA	68	9	
PSA	68	10	
PSM	225	10	
PSM	225	11	
PAP	363	11	
PSM	690	11	
PSM	27	8	
PSM	27	9	
PSM	27	11	
PAP	30	8	
PAP	30	11	
Kalljkrein	138	11	
PSM	592	9	
Kallikrein	222	8	
PSA	218	8	
PSM	603	9	
PSM	603	10	
PSM	660	9	
PSM	660	10	
PSM	660	11	
PSA	56	8	
Kallikrein	60	8	
Kallikrein	53	9	
PSA	49	9	
PAP	262	10	
PSA	134	11	
Kallikrein	192	10	
Kallikrein	192	11	
PSA	188	11	
PSM	352	8	
PSM	352	11	
PSA	8	9	
PSA	8	10	
PSA	8	11	
PSA	1	10	
PSA	1	11	
PSM	394	9	

Table X
Prostate A24 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
Kallikrein	246	8	
PSA	242	8	
PSM	602	10	
PSM	602	11	
Kallikrein	73	8	
Kallikrein	73	9	
PSM	555	11	
PAP	302	9	0.0320
Kallikrein	242	8	
Kallikrein	242	11	
PSM	175	11	
PSA	10	8	
PSA	10	9	
PSM	20	9	
PAP	25	11	
Kallikrein	74	8	
PSM	497	10	
PSA	55	9	
Kallikrein	59	9	
PSM	234	9	
PAP	319	8	
PAP	319	11	
PSM	449	8	
PAP	84	9	
PAP	84	10	
PAP	103	11	
PAP	155	10	
PSM	537	10	
Kallikrein	243	10	
PSA	239	10	
Kallikrein	243	11	
PSA	239	11	
PSM	460	10	
PSM	460	11	
PSM	371	11	
PSM	176	10	
PSM	176	11	
PSM	299	8	
PSM	299	11	
PAP	330	11	

Table XI
Prostate B07 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	B*0702
PSM	236	11	
PSA	14	8	
PSA	14	9	0.0007
PAP	4	8	
PAP	4	9	0.0210
PAP	4	11	
PSM	313	11	
PSM	693	8	
PSM	693	9	0.0003
PAP	351	9	0.0810
PAP	351	10	0.0054
PSM	230	10	0.0002
PAP	56	8	
PSM	677	10	0.0001
PSM	677	11	
PSM	266	9	0.0001
PAP	211	8	
PAP	211	11	
PSM	567	8	
PSM	567	10	0.0001
PSM	567	11	
PSM	387	8	
PSM	387	9	0.0011
PSM	720	9	0.0002
PSA	124	8	
PSA	124	9	0.0001
PSA	124	11	
Kallikrein	128	8	
Kallikrein	128	9	
Kallikrein	128	11	
Kallikrein	145	9	
PSA	141	9	
Kallikrein	145	10	0.0002
PSA	141	10	0.0002
Kallikrein	232	10	
Kallikrein	232	11	
PSA	228	11	
PSM	367	8	
Kallikrein	82	9	
Kallikrein	82	11	
Kallikrein	161	11	
PSA	157	11	
PSM	145	10	0.0001
PSM	705	8	
PSM	705	9	0.0013
PSM	705	11	

Table XI
Prostate B07 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	B*0702
PSA	92	8	
PSA	92	10	1.1000
PSA	92	11	
Kallikrein	96	8	
Kallikrein	96	10	
Kallikrein	96	11	
PAP	124	8	
PAP	124	9	0.0001
PAP	53	11	
PSM	330	8	
Kallikrein	215	8	
PSA	211	8	
Kallikrein	215	9	0.0280
PSA	211	9	0.0280
PAP	361	8	
PSA	78	8	
PSA	78	9	0.0006
PSA	78	11	
PSM	295	8	
PSM	295	11	
PSA	94	8	
PSA	94	9	0.0018
Kallikrein	98	8	
Kallikrein	98	9	
PSM	124	8	
PSM	618	8	
PSM	618	10	0.0003
PSA	184	8	
PSA	184	9	0.1700
PSA	184	10	0.0230
Kallikrein	56	8	
PSA	52	8	
Kallikrein	56	9	0.0240
PSA	52	9	0.0240
PAP	182	8	
PAP	182	10	0.0150
PSM	80	11	
PAP	364	10	0.0019
PAP	277	8	
PAP	277	9	5.8000
PAP	277	10	
PSM	292	8	
PSM	292	9	0.0007
PSM	292	11	
PAP	141	8	
Kallikrein	239	8	

Table XI
Prostate B07 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	B*0702
Kallikrein	239	9	
Kallikrein	239	11	
PSM	681	10	0.0007
PSM	681	11	
Kallikrein	236	8	
Kallikrein	236	11	
PSA	232	8	
PSA	232	11	
PSM	593	8	
PSM	593	9	0.0011
PSM	593	10	0.0150
PSM	593	11	
PAP	156	9	0.0049
PAP	344	10	0.0360
PSM	248	11	
PAP	307	9	0.0029
PSM	289	9	0.0790
PSM	289	11	
PAP	223	10	0.0032
Kallikrein	141	8	
PSA	137	8	
PSM	169	8	
PSM	169	9	0.0001
PSM	169	11	
PAP	133	9	0.0026
PAP	133	11	
PSM	657	8	
PSM	314	10	0.0012
PAP	125	8	
PAP	125	11	
PSM	159	11	
PSM	148	10	0.0001
PSM	148	11	
PSM	147	8	
PSM	147	11	
PSM	146	9	0.0001
PAP	308	8	
PAP	308	11	
PAP	139	8	
PAP	139	10	0.2400
Kallikrein	36	8	
PSA	.32	8	
Kallikrein	112	10	
Kallikrein	112	11	
PSM	684	8	
PSM	684	9	0.4700

Table XI
Prostate B07 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	B*0702
PSM	684	10	0.7200
PSA	108	10	0.0117
PSA	108	11	
PSM	411	8	
PSM	411	9	0.7800
PSM	411	11	
Kallikrein	167	8	
Kallikrein	167	10	
PSM	17	9	0.3200
PSM	17	10	5.2000
PSM	17	11	
PSA	235	8	
PSA	235	9	
PSA	235	11	
PSM	483	11	
PSM	503	10	0.0020
PAP	48	11	
PSM	165	10	0.0002
PSM	165	11	
PAP	348	9	0.0066
PAP	348	10	0.0002
PSM	501	9	0.0025
PSM	269	9	0.0012
PSM	269	10	0.0001
PSM	269	11	
PSM	53	8	
PSM	53	9	0.0990
PSM	53	10	0.0200
PSA	163	8	
PSA	163	10	0.0006
PSM	467	8	
PSM	467	11	
Kallikrein	18	8	
Kallikrein	18	9	
PAP	146	8	
PAP	146	9	0.0002
PAP	146	10	0.0011
PAP	146	11	
Kallikrein	90	11	
PSM	325	9	0.0039
PAP	63	8	
PAP	63	11	
PSM	272	8	
PSM	549	8	
PSM	549	11	
PSM	119	9	0.0001

Table X1
Prostate B07 Supermotif Peptides with Binding Data

Protein	Position	No. of Amino Acids	B*0702
PSM	119	10	0.0035

Table XII
Prostate B27 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
Kallikrein	48	8
PSA	60	9
PSA	60	10
PSA	60	11
Kallikrein	64	10
Kallikrein	64	11
PAP	288	9
PAP	288	11
PSM	111	9
PAP	32	9
PAP	32	10
PAP	32	11
PSM	222	11
Kallikrein	130	9
Kallikrein	130	10
PSM	93	8
PSM	93	11
PAP	9	8
PAP	9	10
PAP	9	11
Kallikrein	185	8
Kallikrein	185	11
PSM	15	9
PSM	15	11
PSM	180	9
PAP	313	8
PSM	597	8
PSM	597	11
PSM	609	8
PSM	654	8
PSM	654	10
PSM	654	11
PSM	683	8
PSM	683	9
PSM	683	10
PSM	683	11
PAP	46	8
PAP	27	9
PAP	27	11
PAP	110	8
PAP	110	11
PSM	563	8
PSM	563	10
PAP	321	9

Table XII
Prostate B27 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PAP	321	10
PAP	321	11
Kallikrein	32	9
PSA	28	9
Kallikrein	32	10
Kallikrein	32	11
PSA	28	10
PSA	28	11
Kallikrein	238	9
Kallikrein	238	10
PAP	254	9
PAP	254	10
PAP	254	11
Kallikrein	190	8
Kallikrein	190	10
PSM	672	8
PSM	672	10
PAP	354	10
PAP	354	11
PSM	444	9
PSA	234	9
PSA	234	10
PSA	77	9
PSA	77	10
PSM	186	9
PSM	570	8
PSM	570	10
PSM	209	9
PSM	209	11
PAP	42	9
PAP	158	10
PSM	376	8
PSM	376	11
PSM	198	8
PSM	198	11
PAP	192	11
PSM	490	8
PSM	206	9
PSM	533	9
PSA	42	8
PSA	42	9
PSA	42	10
PAP	250	9
PSM	377	10

Table XII
Prostate B27 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PAP	249	10
PSM	346	10
PSM	346	11
PAP	58	10
PSM	70	8
PSM	70	11
PSM	43	10
PAP	85	8
PAP	85	9
PSA	63	8
PSA	63	9
PAP	104	10
PAP	104	11
PSM	55	8
PSM	55	11
PSM	617	9
PSM	617	11
Kallikrein	33	8
PSA	29	8
Kallikrein	33	9
Kallikrein	33	10
Kallikrein	33	11
PSA	29	9
PSA	29	10
PSA	29	11
PSM	406	11
PSM	71	10
PAP	281	8
PSA	165	8
PSA	165	10
PSA	165	11
Kallikrein	68	8
PSM	499	8
PSM	499	11
PAP	272	9
PAP	179	9
PAP	179	10
PAP	179	11
PSM	729	8
PSM	729	9
PSM	729	11
PAP	87	11
PSM	5	8
PSM	5	9

Table XII
Prostate B27 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	5	11
PAP	197	9
PAP	197	10
Kallikrein	176	8
Kallikrein	176	10
PAP	181	8
PAP	181	9
PAP	181	11
PSA	172	8
PSA	172	10
PSM	65	9
PSM	65	10
PSM	65	11
PAP	35	8
Kallikrein	67	8
Kallikrein	67	9
PAP	172	10
PSM	481	8
PSM	323	11
PAP	235	9
PAP	235	10
PSM	362	10
PSM	362	11
PSM	604	8
PSM	604	9
PSM	604	11
PSA	120	9
Kallikrein	124	9
PSM	661	8
PSM	661	9
PSM	661	10
PSM	661	11
Kallikrein	111	11
PSA	107	11
Kallikrein	166	9
Kallikrein	166	11
PSM	462	8
PSM	462	9
PSM	462	10
PSM	344	9
PSM	58	8
PSM	58	10
PSM	616	10
PSM	192	9

Table XII
Prostate B27 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	192	10
PSM	192	11
PAP	271	10
PSM	622	8
PSM	622	11
PAP	1	8
PAP	1	9
PAP	1	11
PAP	269	9
PSM	544	8
PSM	544	9
PSM	121	8
PSM	121	10
PSM	121	11
PSM	212	8
PSM	212	9
PSM	212	11
PSM	698	8
PSM	698	11
PSM	81	10
PSA	93	9
PSA	93	10
Kallikrein	97	9
Kallikrein	97	10
PSM	54	8
PSM	54	9
PSA	164	9
PSA	164	11
PAP	162	11
PSM	412	8
PSM	412	10
Kallikrein	168	9
Kallikrein	168	11
PSM	18	8
PSM	18	9
PSM	18	10
PSM	18	11
PAP	336	8
PAP	336	9
PAP	77	8
PAP	77	9
PAP	252	11
PSM	303	11
PAP	178	10

Table XII
Prostate B27 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PAP	178	11
PSA	186	8
PSA	186	1(
PSM	254	8
PSM	254	1:
PSM	526	1(
Kallikrein	88	9
PAP	43	8
PAP	43	1:
PAP	90	8
PAP	86	8
Kallikrein	250	8
PSA	246	8
Kallikrein	250	9
Kallikrein	250	1(
PSA	246	9
PSA	246	1(
PSM	605	8
PSM	605	1(
PSM	280	8
PSM	280	1(
PSM	16	8
PSM	16	1(
PSM	16	11
PSM	413	9
PSM	413	11
Kallikrein	118	9
PSA	114	9
Kallikrein	44	1(
Kallikrein	44	11
PSM	696	1(
Kallikrein	93	8
PSA	89	8
Kallikrein	93	9
PSA	89	9
PSA	89	11
Kallikrein	93	11
PSM	722	11
PSM	644	1(
PSM	513	9
PSM	513	11
PSM	717	8
PSM	717	9
PAP	207	8

Table XII
Prostate B27 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSA	40	10
PSA	40	11
PSM	439	9
PSM	439	10
PSM	439	11
PAP	256	8
PAP	256	9
PSM	123	8
PSM	123	9
PSM	478	11
PSA	189	10
PSM	498	9
PAP	233	9
PAP	233	11
PSM	538	9
Kallikrein	244	9
PSA	240	9
Kallikrein	244	10
PSA	240	10
PSM	353	10
PSM	395	8
PSM	395	11
PAP	218	9
PAP	218	10
PSM	474	8
PSM	294	9
PSA	183	9
PSA	183	10
PSA	183	11
Kallikrein	55	9
PSA	51	9
Kallikrein	55	10
PSA	51	10
PAP	143	11
Kallikrein	247	11
PSA	243	11
PSM	342	11
PSM	214	9
PSM	636	8
PSM	636	11
PSM	728	8
PSM	728	9
PSM	728	10
PSM	239	8

Table XII
Prostate B27 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	239	10
PSM	579	9
PSM	579	10
PSM	100	9
PSM	100	11
PSM	319	9
PSM	319	11
PSM	410	8
PSM	410	9
PSM	410	10
PSM	572	8
PSM	552	8
PSM	552	10
PSM	552	11
PAP	184	8
PAP	184	11
PAP	97	8
PAP	280	9
PAP	89	9
Kallikrein	249	9
PSA	245	9
Kallikrein	249	10
Kallikrein	249	11
PSA	245	10
PSA	245	11
PAP	331	10
PSM	279	8
PSM	279	9
PSM	279	11

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	741	9
PSM	741	10
PSM	742	8
PSM	742	9
PSM	735	8
PSM	735	9
PSA	59	10
PSA	59	11
Kallikrein	63	11
PAP	121	9
PAP	121	11
PSA	13	9
PSA	13	10
PAP	3	9
PAP	3	10
PAP	11	8
PAP	11	9
PAP	11	10
PAP	11	11
PSM	392	8
PSM	392	11
PAP	311	8
PAP	311	9
PAP	311	10
PSM	531	11
PSM	643	8
PSM	643	11
PAP	12	8
PAP	12	9
PAP	12	10
PAP	12	11
PSA	39	11
PSM	419	8
PSM	419	10
PSM	419	11
PSM	13	8
PSM	13	9
PSM	13	11
PAP	227	9
PAP	189	9
PSM	49	10
PAP	274	10
PAP	274	11
PSM	22	8

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	22	11
Kallikrein	234	8
Kallikrein	234	9
Kallikrein	234	10
PSA	230	9
PSA	230	10
PSA	180	9
Kallikrein	184	9
PSA	205	9
PSA	205	10
PSM	196	8
PSM	196	10
PAP	347	10
PAP	347	11
Kallikrein	14	8
PSM	466	8
PSM	466	9
PSM	422	8
PSM	710	10
PSM	301	9
PSA	130	8
Kallikrein	212	11
PSA	208	11
PSM	630	10
Kallikrein	116	8
PSA	112	8
Kallikrein	116	9
PSA	112	9
Kallikrein	116	11
PSA	112	11
PSM	453	8
PSM	453	10
PSM	316	8
PSM	316	10
PSM	106	8
PSM	106	10
PSM	106	11
PSM	379	8
Kallikrein	207	11
PAP	51	8
Kallikrein	85	8
PSA	81	8
PAP	230	9
PAP	290	9

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PAP	290	10
PAP	290	11
PSM	48	11
PSM	285	8
PSM	285	10
PAP	168	10
PSM	703	9
PSM	703	10
PSM	703	11
PSM	716	9
PSM	716	10
PAP	60	8
PAP	60	11
PAP	216	8
PAP	216	11
PAP	95	8
PAP	95	9
PAP	95	10
PSM	7	9
PAP	170	8
PSM	542	8
PSM	542	10
PSM	542	11
PAP	334	9
PAP	334	10
PAP	334	11
PSM	557	9
PSM	557	10
PAP	356	8
PAP	356	9
PSM	235	8
PSM	418	9
PSM	418	11
PSM	161	9
PSM	633	10
PSM	633	11
PSM	646	8
PSM	506	10
PSM	546	10
PSM	546	11
PSM	164	11
PSM	337	8
PSM	337	10
PSM	639	8

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	333	10
PSM	77	8
PSM	737	10
PSA	12	10
PSA	12	11
PSM	391	8
PSM	391	9
PSM	263	10
PSM	221	8
PSM	24	9
PSM	24	10
PSM	24	11
PSM	364	8
PSM	364	9
PSM	364	10
PSM	364	11
Kallikrein	16	10
Kallikrein	16	11
PSM	311	9
PSM	516	8
PSM	516	9
PSM	516	10
Kallikrein	158	8
PSA	154	8
Kallikrein	158	9
PSA	154	9
PSM	321	9
PSM	85	8
PSM	85	9
PSM	403	8
Kallikrein	149	9
PSA	145	9
Kallikrein	94	8
PSA	90	8
PSA	90	10
Kallikrein	94	10
Kallikrein	34	8
Kallikrein	34	9
Kallikrein	34	10
PSA	30	8
PSA	30	9
PSA	30	10
PSM	347	9
PSM	347	10

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	553	9
PSM	553	10
PAP	144	10
PAP	144	11
PSM	283	10
Kallikrein	8	10
Kallikrein	8	11
PSM	202	8
PSM	530	8
PSM	642	9
PAP	188	10
PSM	128	11
PSM	512	10
PSM	614	9
PSA	175	9
Kallikrein	132	8
Kallikrein	132	10
PSM	52	9
PSM	52	10
PSM	52	11
Kallikrein	226	10
Kallikrein	226	11
PSA	222	10
PSA	222	11
PSM	66	8
PSM	66	9
PSM	66	10
PSM	59	9
PSM	723	10
PSM	723	11
PAP	173	9
PSM	655	9
PSM	655	10
PSM	500	10
PAP	255	8
PAP	255	9
PAP	255	10
PSM	44	9
PSA	66	11
PSM	240	9
PSM	122	9
PSM	122	10
PSM	623	10
PSM	623	11

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PAP	120	10
PSM	219	8
PSM	219	9
PSM	219	10
PSM	28	8
PSM	28	10
PSM	28	11
PSM	83	8
PSM	83	10
PSM	83	11
PSM	110	8
PSM	110	10
PAP	31	8
PAP	31	10
PAP	31	11
PSM	92	9
PSM	587	9
PAP	8	9
PAP	8	11
PAP	148	8
PAP	148	9
PAP	148	11
PAP	238	9
PAP	238	10
PSA	122	10
PSA	122	11
Kallikrein	126	10
Kallikrein	126	11
PAP	194	9
PAP	194	10
PAP	14	8
PAP	14	9
PAP	14	10
PAP	14	11
PAP	241	9
Kallikrein	179	9
Kallikrein	179	10
PSA	18	8
Kallikrein	10	8
Kallikrein	10	9
Kallikrein	10	11
PSA	6	8
PSA	6	9
PSA	6	11

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	117	11
PSA	128	8
PSA	128	10
PAP	315	11
PSA	4	8
PSA	4	10
PSA	4	11
PSM	268	10
PSM	268	11
PSA	162	9
PSA	162	11
PAP	70	10
PSM	574	10
PSM	574	11
PAP	298	9
PAP	298	10
PAP	114	8
PAP	114	9
PAP	114	10
PAP	114	11
Kallikrein	103	9
PSA	99	8
PSA	99	9
PAP	232	10
PAP	117	8
PSM	451	10
PSM	216	10
PSM	216	11
Kallikrein	70	11
PSM	438	10
PSM	438	11
PSM	231	9
PSA	125	8
PSA	125	10
PSA	125	11
Kallikrein	129	8
Kallikrein	129	10
Kallikrein	129	11
Kallikrein	146	8
PSA	142	8
Kallikrein	146	9
PSA	142	9
PSM	273	11
Kallikrein	240	8

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
Kallikrein	240	10
PAP	349	8
PAP	349	9
PAP	349	11
PSM	290	8
PSM	290	10
PSM	290	11
PSM	721	8
PSA	236	8
PSA	236	10
PSM	502	8
PSM	502	11
PSM	694	8
PAP	224	9
PAP	278	8
PAP	278	9
PAP	278	11
PAP	54	10
PSM	740	10
PSM	740	11
PSM	389	10
PSM	389	11
PSM	97	9
Kallikrein	22	8
PAP	2	8
PAP	2	10
PAP	2	11
PAP	10	9
PAP	10	10
PAP	10	11
PSM	673	9
PSM	534	8
PAP	273	8
PAP	273	11
PSA	43	8
PSA	43	9
Kallikrein	186	10
Kallikrein	186	11
PSM	400	11
Kallikrein	169	8
Kallikrein	169	10
Kallikrein	169	11
PAP	105	9
PAP	105	10

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PAP	28	8
PAP	28	10
PAP	28	11
PSM	181	8
PSM	414	8
PSM	414	10
PAP	111	10
PAP	111	11
PSM	162	8
PAP	287	10
PAP	115	8
PAP	115	9
PAP	115	10
PSM	312	8
PSM	10	11
PSM	634	9
PSM	634	10
Kallikrein	117	8
PSA	113	8
Kallikrein	117	10
PSA	113	10
PSM	695	11
PSM	454	9
PSM	454	11
PSM	45	8
PAP	61	10
PSM	317	9
PSM	317	11
PSA	203	11
PAP	106	8
PAP	106	9
PAP	106	11
PSM	431	11
PSM	348	8
PSM	348	9
PSM	348	11
PSM	338	9
PSA	58	11
PSM	14	8
PSM	14	10
PSM	141	11
Kallikrein	227	9
Kallikrein	227	10
PSA	223	9

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSA	223	10
Kallikrein	150	8
PSA	146	8
Kallikrein	150	11
PSA	146	11
PAP	291	8
PAP	291	9
PAP	291	10
PSM	734	8
PSM	734	9
PSM	734	10
PSM	576	8
PSM	576	9
PSM	576	10
PSA	38	8
PSM	12	9
PSM	12	10
Kallikrein	40	8
Kallikrein	40	9
PSM	447	10
PSM	154	8
PSM	154	10
PSM	154	11
PSM	627	9
PSM	627	10
PAP	293	8
PAP	293	10
PAP	293	11
Kallikrein	92	9
PSA	88	9
Kallikrein	92	10
PSA	88	10
PAP	129	8
PAP	129	9
PAP	129	10
Kallikrein	174	10
Kallikrein	192	8
Kallikrein	192	10
Kallikrein	192	11
PSA	188	8
PSA	188	11
PSM	352	8
PSM	352	11
PSA	8	9

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSA	8	10
PSA	8	11
PSM	434	8
PSM	434	9
Kallikrein	47	8
Kallikrein	47	9
PAP	226	10
PAP	206	8
PAP	206	9
PSM	497	10
PSM	607	8
PSM	607	10
PSM	700	9
PSM	700	10
PSM	692	9
PSM	692	10
PSM	179	8
PSM	179	10
PAP	310	9
PAP	310	10
PAP	310	11
Kallikrein	153	8
PSA	149	8
PSM	600	8
PSM	600	9
PSM	277	8
PSM	277	10
PSM	277	11
PAP	286	8
PAP	286	11
PSM	228	8
PSM	228	9
Kallikrein	188	8
Kallikrein	188	9
Kallikrein	188	10
Kallikrein	43	11
PSM	612	11
PSM	471	11
PSM	625	8
PSM	625	9
PSM	625	11
PSM	537	10
Kallikrein	243	10
PSA	239	10

Table XIII
Prostate B58 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
Kallikrein	243	11
PSA	239	11
PSM	460	10
PSM	460	11

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PAP	299	8
PAP	299	9
PSM	711	9
PAP	122	8
PAP	122	10
PAP	122	11
Kallikrein	147	8
PSA	143	8
Kallikrein	147	11
PSA	143	11
Kallikrein	235	8
Kallikrein	235	9
PSA	231	8
PSA	231	9
Kallikrein	9	9
Kallikrein	9	10
PSM	25	8
PSM	25	9
PSM	25	10
PSM	25	11
PAP	116	8
PAP	116	9
PSM	236	11
PSA	14	8
PSA	14	9
PAP	4	8
PAP	4	9
PAP	4	11
PSM	313	11
PSM	693	8
PSM	693	9
PSM	302	8
PSM	217	9
PSM	217	10
PSM	217	11
PSA	181	8
PSA	181	11
PSM	577	8
PSM	577	9
PSM	577	11
PSM	11	10
PSM	11	11
PSA	44	8
PSM	365	8

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	365	9
PSM	365	10
PSM	286	9
PSM	635	8
PSM	635	9
Kallikrein	17	9
Kallikrein	17	10
PSM	393	10
PSM	601	8
PSM	601	11
Kallikrein	41	8
Kallikrein	241	9
PSA	62	8
PSA	62	9
PSA	62	10
Kallikrein	66	8
Kallikrein	66	9
Kallikrein	66	10
PAP	351	9
PAP	351	10
PSA	169	11
Kallikrein	173	11
PSM	714	11
PSM	156	8
PSM	156	9
PAP	201	9
PAP	201	10
PSA	171	9
PSA	171	11
Kallikrein	120	11
PSA	116	11
PSA	136	8
PSA	136	9
Kallikrein	3	8
Kallikrein	3	10
PSM	173	8
Kallikrein	182	11
PSM	191	10
PSM	191	11
PSA	98	9
PSA	98	10
PSM	230	10
PAP	56	8
PSM	677	10

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	677	11
PSM	266	9
PAP	211	8
PAP	211	11
PSM	567	8
PSM	567	10
PSM	567	11
PSM	387	8
PSM	387	9
PSM	720	9
PAP	151	8
PSM	666	9
PSM	666	10
PSM	666	11
PSA	178	11
PAP	108	9
PAP	108	10
Kallikrein	134	8
PAP	301	10
PAP	301	11
PSM	641	10
PSM	137	8
PAP	266	9
PSM	397	9
PSM	109	8
PSM	109	9
PSM	109	11
PSM	586	8
PSM	586	10
PAP	80	10
PSM	64	10
PSM	64	11
PAP	34	8
PAP	34	9
PSM	480	9
PAP	237	8
PAP	237	10
PAP	237	11
PAP	240	8
PAP	240	10
PSA	127	8
PSA	127	9
PSA	127	11
PSM	560	10

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	560	11
PAP	358	11
PAP	317	9
PAP	317	10
PAP	317	11
PSM	621	9
PSA	124	8
PSA	124	9
PSA	124	11
Kallikrein	128	8
Kallikrein	128	9
Kallikrein	128	11
Kallikrein	145	9
PSA	141	9
Kallikrein	145	10
PSA	141	10
Kallikrein	232	10
Kallikrein	232	11
PSA	228	11
PSM	367	8
Kallikrein	82	9
Kallikrein	82	11
Kallikrein	161	11
PSA	157	11
PSM	145	10
PAP	76	9
PAP	76	10
PSM	87	10
PAP	100	10
PSM	522	9
PSM	522	10
PSM	727	8
PSM	727	9
PSM	727	10
PSM	727	11
PSM	351	8
PSM	351	9
PAP	187	8
PAP	187	11
PSM	42	8
PSM	42	11
PSM	61	10
PSM	670	10
PAP	18	8

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PAP	18	9
PAP	20	11
PSM	33	9
PSM	33	10
PSM	33	11
PAP	92	11
Kallikrein	165	10
PSA	3	8
PSA	3	9
PSA	3	11
PSA	161	10
PSM	73	8
PSM	73	11
Kallikrein	195	8
PSA	191	8
PSM	705	8
PSM	705	9
PSM	705	11
PSA	92	8
PSA	92	10
PSA	92	11
Kallikrein	96	8
Kallikrein	96	10
Kallikrein	96	11
PAP	124	8
PAP	124	9
PAP	53	11
PAP	164	9
PAP	177	8
PAP	177	11
PSM	90	11
PSM	525	11
PSA	86	11
PSM	282	8
PSM	282	11
PSM	529	9
PSM	385	8
PSM	385	9
PSM	385	10
PSM	385	11
PAP	248	11
Kallikrein	225	11
PSA	221	11
PAP	204	10

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PAP	204	11
PSM	707	9
PSM	104	8
PSM	104	10
PAP	196	8
PAP	196	10
PAP	196	11
PSM	427	8
PSM	427	9
PAP	305	11
PSM	680	8
PSM	680	11
PSM	288	10
Kallikrein	140	8
Kallikrein	140	9
PAP	295	8
PAP	295	9
PAP	74	8
PAP	74	11
PSM	168	8
PSM	168	9
PSM	168	10
PSM	508	8
PSM	582	10
PSM	582	11
PSM	330	8
Kallikrein	215	8
PSA	211	8
Kallikrein	215	9
PSA	211	9
PAP	361	8
PAP	199	8
PAP	199	11
PAP	68	8
Kallikrein	87	10
PSA	83	10
PSM	446	11
PSM	224	9
PSM	224	11
PSM	238	9
PSM	238	11
Kallikrein	221	9
PSA	217	9
Kallikrein	52	8

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSA	48	8
Kallikrein	52	9
PSA	48	9
Kallikrein	52	10
PSA	48	10
PAP	261	8
PAP	261	11
PSM	252	8
PSM	252	10
PAP	128	8
PAP	128	9
PAP	128	10
PAP	128	11
PSM	345	8
PSM	345	11
PSM	82	9
PSM	82	11
Kallikrein	177	9
Kallikrein	177	11
PSM	573	11
PAP	270	8
PAP	270	11
PSA	78	8
PSA	78	9
PSA	78	11
PSM	295	8
PSM	295	11
PSA	94	8
PSA	94	9
Kallikrein	98	8
Kallikrein	98	9
PSM	124	8
PSM	618	8
PSM	618	10
PSA	184	8
PSA	184	9
PSA	184	10
Kallikrein	56	8
PSA	52	8
Kallikrein	56	9
PSA	52	9
PAP	182	8
PAP	182	10
PSA	173	9

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSA	173	11
PSM	130	9
PSM	130	10
PSM	416	8
PSM	416	11
PSM	373	9
PSM	373	10
PSM	373	11
PSA	69	8
PSA	69	9
PAP	135	9
PAP	267	8
PAP	267	11
PSM	258	11
PSA	17	9
PSM	226	9
PSM	226	10
PSM	226	11
PAP	284	10
PSM	80	11
PAP	364	10
PAP	277	8
PAP	277	9
PAP	277	10
PSM	292	8
PSM	292	9
PSM	292	11
PAP	141	8
PSM	96	8
PSM	96	10
Kallikrein	21	9
PSM	200	9
PSM	200	10
PSM	591	10
PSM	591	11
PSM	659	10
PSM	659	11
PSM	157	8
PSM	398	8
PSM	193	8
PSM	193	9
PSM	193	10
PSM	193	11
Kallikrein	131	8

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
Kallikrein	131	9
Kallikrein	131	11
PSM	199	10
PSM	199	11
PSM	187	8
PSM	514	8
PSM	514	10
PSM	514	11
PSM	304	10
PSA	166	9
PSA	166	10
PAP	234	8
PAP	234	10
PAP	234	11
PAP	193	10
PAP	193	11
PSM	343	10
Kallikrein	239	8
Kallikrein	239	9
Kallikrein	239	11
PSM	94	10
PAP	251	8
PSM	718	8
PSM	718	11
PSM	207	8
PSM	207	11
PSM	213	8
PSM	213	10
Kallikrein	137	11
PSA	133	11
PSM	324	10
Kallikrein	191	9
Kallikrein	191	11
PSA	187	9
Kallikrein	245	8
PSA	241	8
Kallikrein	245	9
PSA	241	9
PAP	208	11
PSA	16	10
PAP	283	11
Kallikrein	20	10
PAP	7	8
PAP	7	10

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	305	9
PAP	21	10
PAP	21	11
PSM	34	8
PSM	34	9
PSM	34	10
PSA	70	8
PSM	428	8
PSM	4	8
PSM	4	9
PSM	4	10
PAP	6	9
PAP	6	11
PAP	306	10
PSM	441	8
PSM	441	9
PSM	441	10
Kallikrein	123	8
PSA	119	8
PSA	119	10
Kallikrein	123	10
Kallikrein	178	8
Kallikrein	178	10
Kallikrein	178	11
PAP	136	8
PAP	136	11
PSM	668	8
PSM	668	9
Kallikrein	121	10
PSA	117	10
PAP	113	8
PAP	113	9
PAP	113	10
PAP	113	11
PSM	469	9
PSM	681	10
PSM	681	11
Kallikrein	236	8
Kallikrein	236	11
PSA	232	8
PSA	232	11
PSM	593	8
PSM	593	9
PSM	593	10

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	593	11
PAP	156	9
PAP	344	10
PSM	248	11
PAP	307	9
PSM	289	9
PSM	289	11
PAP	223	10
Kallikrein	141	8
PSA	137	8
PSA	167	8
PSA	167	9
Kallikrein	171	8
Kallikrein	171	9
PSM	650	10
PSM	650	11
PSM	442	8
PSM	442	9
PSM	442	11
PAP	258	10
PAP	258	11
PAP	296	8
PAP	296	11
PSA	37	8
PSA	37	9
Kallikrein	217	10
PSA	213	10
PSM	561	9
PSM	561	10
PAP	40	11
PAP	359	10
PSM	473	9
Kallikrein	54	8
PSA	50	8
Kallikrein	54	10
PSA	50	10
Kallikrein	54	11
PSA	50	11
PSM	26	8
PSM	26	9
PSM	26	10
Kallikrein	4	9
PAP	263	9
Kallikrein	122	9

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSA	118	9
PSA	118	11
Kallikrein	122	11
PAP	343	11
PSM	663	8
PSM	663	9
PSM	169	8
PSM	169	9
PSM	169	11
PSM	583	9
PSM	583	10
PSM	583	11
PSM	69	9
PSM	257	8
PSM	51	8
PSM	51	10
PSM	51	11
PAP	119	11
PSM	3	9
PSM	3	10
PSM	3	11
PSM	260	9
PSM	57	9
PSM	57	11
Kallikrein	102	10
PAP	133	9
PAP	133	11
PSM	657	8
PSM	328	10
PSM	357	9
PSM	357	10
PSM	153	9
PSM	153	11
PAP	49	10
PSM	296	10
PSM	296	11
PAP	57	11
PAP	134	8
PAP	134	10
PAP	140	9
PSM	658	11
PAP	352	8
PAP	352	9
PSM	678	9

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	678	10
PSA	15	8
PSA	15	11
Kallikrein	19	8
Kallikrein	19	11
PAP	5	8
PAP	5	10
PSM	468	10
PAP	147	8
PAP	147	9
PAP	147	10
PSM	267	8
PSM	267	11
Kallikrein	216	8
PSA	212	8
Kallikrein	216	11
PSA	212	11
PAP	212	10
PSA	95	8
PSM	550	10
Kallikrein	99	8
PSM	568	9
PSM	568	10
PSM	314	10
PAP	125	8
PAP	125	11
PSM	159	11
PSM	148	10
PSM	148	11
PSM	147	8
PSM	147	11
PSM	146	9
PAP	308	8
PAP	308	11
PAP	365	9
PSM	619	9
PSM	619	11
PAP	64	10
PSM	166	9
PSM	166	10
PSM	166	11
PSA	185	8
PSA	185	9
PSA	185	11

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	388	8
PSM	388	11
Kallikrein	57	8
PSA	53	8
PSA	53	11
Kallikrein	57	11
PSM	293	8
PSM	293	10
Kallikrein	91	10
Kallikrein	91	11
PAP	276	8
PAP	276	9
PAP	276	10
PAP	276	11
PSM	95	9
PSM	95	11
PSM	731	9
PSM	731	11
PSM	218	8
PSM	218	9
PSM	218	10
PSM	218	11
PSM	91	10
PAP	72	8
PAP	72	10
PSM	667	8
PSM	667	9
PSM	667	10
PAP	69	11
PAP	297	10
PAP	297	11
PAP	139	8
PAP	139	10
Kallikrein	36	8
PSA	32	8
Kallikrein	39	9
Kallikrein	39	10
PSA	84	9
PSA	182	10
PSA	182	11
PSA	35	10
PSA	35	11
PSM	578	8
PSM	578	10

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	578	11
PSA	87	10
PSA	87	11
Kallikrein	72	9
Kallikrein	72	10
PAP	101	9
PSM	511	11
PSM	354	9
PSM	527	9
PSM	527	11
PAP	180	8
PAP	180	9
PAP	180	10
PSM	440	8
PSM	440	9
PSM	440	10
PSM	440	11
PSM	649	11
PAP	257	8
PAP	257	11
PSA	121	8
PSA	121	11
Kallikrein	125	8
Kallikrein	125	11
PSM	662	8
PSM	662	9
PSM	662	10
Kallikrein	112	10
Kallikrein	112	11
PSM	684	8
PSM	684	9
PSM	684	10
PSA	108	10
PSA	108	11
PSM	411	8
PSM	411	9
PSM	411	11
Kallikrein	167	8
Kallikrein	167	10
PSM	17	9
PSM	17	10
PSM	17	11
PSA	235	8
PSA	235	9

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSA	235	11
PSM	730	8
PSM	730	10
PSM	463	8
PSM	463	9
PSM	463	11
Kallikrein	89	8
Kallikrein	7	11
PSM	455	8
PSM	455	10
Kallikrein	159	8
PSA	155	8
PSM	129	10
PSM	129	11
PSM	291	9
PSM	291	10
PSM	613	10
PSM	590	11
PAP	130	8
PAP	130	9
PSM	142	10
PSA	75	11
PSM	631	9
PAP	15	8
PAP	15	9
PAP	15	10
PAP	15	11
Kallikrein	175	9
Kallikrein	175	11
PSM	322	8
Kallikrein	104	8
PSA	100	8
PAP	242	8
Kallikrein	170	9
Kallikrein	170	10
PAP	13	8
PAP	13	9
PAP	13	10
PAP	13	11
PSM	472	10
PSA	237	9
PSM	615	8
PSM	615	11
PSM	483	11

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	503	10
PAP	48	11
PSM	165	10
PSM	165	11
PAP	348	9
PAP	348	10
PSM	501	9
Kallikrein	35	8
Kallikrein	35	9
PSA	31	8
PSA	31	9
Kallikrein	71	10
Kallikrein	71	11
PSM	98	8
PSM	98	11
PSM	107	9
PSM	107	10
PSM	107	11
Kallikrein	11	8
Kallikrein	11	10
Kallikrein	11	11
PAP	217	10
PAP	217	11
PSA	67	10
PSA	67	11
PAP	29	9
PAP	29	10
PSM	626	8
PSM	626	10
PSM	626	11
PSA	7	8
PSA	7	10
PSA	7	11
PSM	554	8
PSM	554	9
PSM	415	9
PAP	190	8
PAP	171	11
PAP	112	9
PAP	112	10
PAP	112	11
PAP	222	11
PSM	361	11
PSM	461	9

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSA	68	10
PSM	225	8
PSM	225	10
PSM	225	11
PAP	363	11
PSA	174	8
PSA	174	10
PSM	690	11
PSM	27	8
PSM	27	9
PSM	27	11
PAP	30	8
PAP	30	9
PAP	30	11
Kallikrein	138	10
Kallikrein	138	11
PSM	592	9
PSM	592	10
PSM	592	11
Kallikrein	222	8
PSA	218	8
PSM	603	9
PSM	603	10
PSM	660	9
PSM	660	10
PSM	660	11
Kallikrein	5	8
PSA	56	8
Kallikrein	60	8
PSA	36	9
PSA	36	10
Kallikrein	53	8
PSA	49	8
Kallikrein	53	9
PSA	49	9
Kallikrein	53	11
PSA	49	11
PAP	262	10
PSA	134	10
PSA	134	11
Kallikrein	18	8
Kallikrein	18	9
PAP	146	8
PAP	146	9

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	461	10
PSM	461	11
PSA	5	9
PSA	5	10
PAP	231	8
PAP	231	11
PSM	269	9
PSM	269	10
PSM	269	11
PSM	53	8
PSM	53	9
PSM	53	10
PSA	163	8
PSA	163	10
PSM	467	8
PSM	467	11
Kallikrein	143	11
PSA	139	11
PAP	335	8
PAP	335	9
PAP	335	10
PAP	275	9
PAP	275	10
PAP	275	11
PSM	339	8
PAP	71	9
PAP	71	11
PSM	575	9
PSM	575	10
PSM	575	11
PAP	145	9
PAP	145	10
PAP	145	11
PSM	738	9
PAP	292	8
PAP	292	9
PAP	292	11
PSM	201	8
PSM	201	9
PSM	358	8
PSM	358	9
PSM	372	10
PSM	372	11
PSA	68	9

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PAP	146	10
PAP	146	11
Kallikrein	90	11
PSM	325	9
PSM	739	8
PSM	739	11
PSM	253	9
PSA	1	8
PSA	1	10
PSA	1	11
PSM	394	9
Kallikrein	246	8
PSA	242	8
PSM	602	10
PSM	602	11
PSA	10	8
PSA	10	9
Kallikrein	252	8
PSA	248	8
PSM	20	8
PSM	20	9
PSM	20	10
PAP	25	8
PAP	25	11
Kallikrein	74	8
PAP	63	8
PAP	63	11
PAP	138	9
PAP	138	11
Kallikrein	38	10
Kallikrein	38	11
PSA	34	11
PSA	55	9
Kallikrein	59	9
PSM	449	8
PAP	84	9
PAP	84	10
PAP	103	11
PAP	155	10
PSM	272	8
PSM	549	8
PSM	549	11
PSM	119	9
PSM	119	10

Table XIV
Prostate B62 Supermotif with Binding Data

Protein	Position	No. of Amino Acids
PSM	733	9
PSM	733	10
PSM	733	11
PSM	371	11
PSM	176	10
PSM	176	11

Table XV
Prostate A01 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0101
PSM	452	9	
PSM	220	9	
PSM	264	9	0.0099
PSM	701	9	0.0040
PSM	693	8	
PAP	311	9	0.7700
PSM	597	11	
PSM	196	10	0.0160
PSM	453	8	
PSM	106	8	
PSM	599	9	
PSM	171	9	0.0024
PSM	109	11	
PAP	237	11	
PAP	240	8	
Kallikrein	145	9	0.0011
PSA	141	9	0.0011
PAP	95	9	0.0980
PSM	542	8	
PSM	542	11	
PSM	557	10	0.0260
PSM	546	11	
PSM	565	8	
PSM	702	8	
PSM	487	8	
PSM	529	9	0.0025
PSM	104	10	0.4800
PAP	74	11	
PSM	168	9	0.0001
PAP	270	11	
Kallikrein	94	8	0.0260
PSA	90	8	0.0260
Kallikrein	34	10	
PSM	347	10	0.0048
PSM	112	8	
PSM	530	8	
PSM	346	11	
PSM	450	11	
PAP	277	10	0.5700
PAP	205	10	0.0012
PSM	691	10	
PSM	66	10	0.0001
PSM	545	8	
PAP	322	9	3.4000
PAP	322	10	0.0180
Kallikrein	33	11	

Table XV
Prostate A01 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0101
Kallikrein	239	11	
PAP	272	9	0.0011
PSM	699	11	
PSM	105	9	
PSM	143	9	0.0010
PAP	81	9	0.7800
PSM	65	11	
Kallikrein	178	11	
PAP	93	11	
Kallikrein	236	8	
PSA	232	8	0.0002
PSM	289	11	
PSM	442	8	
PAP	148	8	
PAP	238	10	12.0000
Kallikrein	179	10	
PSM	117	11	
PAP	315	11	
PSM	268	10	0.0082
PAP	70	10	0.6200
PSM	227	8	
PSM	169	8	
PSM	169	11	
PSM	451	10	0.4300
PSM	195	11	
PAP	94	10	0.0033
PSM	262	11	
PSM	540	10	
Kallikrein	233	11	
PSA	229	11	
PSM	484	11	
PAP	147	9	1.2000
PSM	290	10	
PSM	290	11	
PSA	236	10	0.0010
PAP	278	9	0.0031
Kallikrein	91	11	
PAP	309	11	
PSM	218	11	
PSA	87	11	
PSM	363	9	0.0001
PSM	320	8	
PAP	332	9	0.0002
PSA	235	11	
PSM	463	9	11.0000
PAP	174	11	

Table XV
Prostate A01 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0101
Kallikrein	93	9	0.0011
PSA	89	9	0.0011
PSM	615	11	
Kallikrein	180	9	
PSM	317	11	
PSM	348	9	0.0430
PSM	349	8	
Kallikrein	143	11	0.0190
PSA	139	11	0.0190
PSM	141	11	
PSM	558	9	0.0010
PAP	293	11	
Kallikrein	92	10	0.1500
PSA	88	10	0.1500
PSM	725	9	0.0010
PAP	206	9	0.0046
PAP	310	10	0.5500
PSM	234	9	
PSM	552	8	
PSM	272	8	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	741	10	
PSM	742	9	
PSM	735	8	
PSM	735	9	
PSA	59	8	
PSA	13	8	
PAP	3	8	
PAP	3	9	
PAP	3	10	
PAP	11	8	
PAP	11	10	
PSM	392	9	
PSM	392	11	
PSM	608	10	
PSM	608	11	
PSM	452	9	
PSM	232	9	0.0006
PSM	232	11	
PSM	674	11	
PSM	60	8	
PSM	736	8	
PSM	220	9	
PSM	23	10	
PSM	23	11	
PSM	264	9	
PSM	264	11	
PSM	701	9	
PSM	701	11	
PSM	29	9	
PSM	29	11	
Kallikrein	199	8	
PSA	195	8	
PSM	84	10	
PSM	84	11	
PSM	711	8	
Kallikrein	147	8	
PSA	143	8	
Kallikrein	235	9	
Kallikrein	235	11	
PSA	231	9	0.0170
PSA	231	11	
Kallikrein	9	9	
PSM	25	8	
PSM	25	9	
PAP	116	9	
PAP	311	9	0.0002

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PAP	311	10	
PSM	531	9	0.0086
PSM	643	11	
PAP	12	9	
PSM	419	8	
PSM	13	11	
PAP	227	8	0.0003
PAP	227	10	
PAP	189	10	
PSM	49	8	
PSM	49	11	
PAP	274	8	0.0180
PAP	274	9	0.1000
PSM	11	9	
PSA	44	9	
PSM	286	10	
PSM	635	9	
PSM	635	11	
Kallikrein	17	8	
PSM	393	8	
PSM	393	10	
PSM	601	8	
PSM	601	10	0.0026
Kallikrein	41	8	
Kallikrein	41	9	
Kallikrein	241	8	
Kallikrein	241	9	
Kallikrein	241	10	
Kallikrein	241	11	
PSM	22	8	
PSM	22	11	
Kallikrein	198	9	
PSA	194	9	0.0006
Kallikrein	234	8	
Kallikrein	234	10	
PSA	230	10	
PSA	180	8	
PSA	180	11	
Kallikrein	184	8	
PSM	196	8	
PSM	196	9	
PSM	196	10	0.0600
PAP	347	9	0.0040
PAP	347	10	
PAP	347	11	
Kallikrein	14	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	466	10	
PSM	710	9	0.0006
PSM	301	8	
PSM	596	10	
PSM	596	11	
PSM	465	11	
PSA	111	11	
PSM	652	11	
PSM	520	8	
PSM	184	10	
PAP	186	8	
PSM	134	11	
PSM	714	10	0.0003
PSM	714	11	
PSM	156	8	
PSM	156	9	
PAP	201	8	
PAP	201	10	
PSA	171	11	
Kallikrein	120	11	
PSA	116	11	
PSA	136	8	
PSM	173	8	
PSM	173	9	
Kallikrein	182	10	
PSM	191	9	
PSA	98	8	0.0003
PSA	98	9	
PSA	98	11	
PSM	9	8	
PSM	9	9	
PSM	9	11	
PSM	630	8	
PSM	630	10	
Kallikrein	116	10	
PSA	112	10	
PSM	453	8	
PSM	453	11	
PSM	316	9	0.0032
PSM	106	8	
PAP	51	9	0.0001
Kallikrein	85	10	
PSA	81	10	
PAP	290	10	
PSA	178	10	0.0007
PAP	108	9	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	114	9	0.0006
PSM	114	11	
PAP	301	10	
PAP	301	11	
PSM	48	8	
PSM	48	9	
PSM	285	11	
PAP	371	8	
PSM	183	8	
PSM	183	11	
PAP	150	9	
PAP	150	10	
Kallikrein	115	11	
Kallikrein	84	11	
PSA	80	11	
PAP	229	8	
PSM	102	10	
PSM	102	11	
PSM	425	11	
PAP	176	9	
PAP	176	10	
PSM	505	10	
PSM	171	9	
PSM	171	10	
PSM	171	11	
PSM	486	9	
PSM	489	11	
PSM	408	11	
PSM	641	9	0.0006
PSM	137	8	
PAP	266	8	
PAP	266	9	
PSM	397	10	
PSM	397	11	
PSM	109	11	
PSM	586	10	
PAP	166	8	
PAP	80	8	
PAP	80	9	
PAP	80	10	
PAP	80	11	
PSM	64	8	
PSM	64	9	
PSM	64	10	
PAP	34	9	
PAP	34	10	0.0014

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PAP	23	11	
PSM	383	10	
PSM	383	11	
PAP	203	8	
PSM	103	9	
PSM	103	10	
PSM	103	11	
PSM	426	10	
PSM	402	10	
PSM	39	11	
PSM	675	10	
PSM	42	8	
PSM	61	11	
PSM	37	8	
PAP	18	11	
PAP	20	9	0.0024
PSM	33	10	
PAP	92	8	
PSA	106	10	
PSA	3	11	
PSM	73	10	0.0102
PSM	633	11	
PSM	646	8	
PSM	646	10	0.0003
PSM	506	9	
PSM	546	8	
PSM	546	11	
PSM	337	9	
PSM	337	11	
PSM	639	8	
PSM	639	11	
PSM	333	9	
PSM	333	11	
PSM	77	8	
PAP	37	8	
PAP	37	11	
PSA	12	9	0.0150
PSM	391	10	
PSM	263	10	
PSM	221	8	
PSM	24	9	
PSM	24	10	
PSM	364	8	
Kallikrein	16	9	
PAP	346	10	
PAP	346	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	172	8	
PSM	172	9	
PSM	172	10	
PSM	265	8	
PSM	265	10	
PAP	45	9	
PSM	487	8	
PSM	31	9	0.0005
PSM	36	9	0.0007
PAP	17	8	
PSM	332	10	
PSM	30	8	
PSM	30	10	
PSM	375	9	
PSM	384	9	
PSM	384	10	
PSM	581	8	
PSM	310	11	
PAP	260	11	
Kallikrein	27	8	
PSA	23	8	
PSM	529	8	
PSM	529	9	
PSM	529	11	
PSM	385	8	
PSM	385	9	
PAP	248	8	
PAP	248	10	
Kallikrein	225	11	
PSA	221	11	
PAP	204	11	
PSM	104	8	
PSM	104	9	
PSM	104	10	
PAP	196	8	
PSM	427	9	
PAP	305	10	
PSM	680	8	
PSM	680	9	0.0460
PSM	680	10	
PSM	288	8	
Kallikrein	140	8	
PAP	295	9	
PAP	74	11	
PSM	168	9	0.0007
PSM	311	10	0.0006

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSA	226	10	
PSM	516	9	
PSM	516	10	
Kallikrein	158	8	
PSA	154	8	
Kallikrein	158	10	
PSM	430	11	
PSM	85	9	
PSM	85	10	
PSM	403	9	
PSM	403	11	
PSM	360	11	
PSM	224	9	
PSM	224	11	
PAP	261	10	
Kallikrein	49	8	
PAP	289	11	
PAP	44	10	
PAP	198	11	
PSM	345	10	
PSM	82	9	
Kallikrein	177	9	
Kallikrein	177	10	
Kallikrein	177	11	
PAP	314	9	0.2700
PSM	573	8	
PAP	270	11	
Kallikrein	94	8	0.0890
PSA	90	8	0.0890
Kallikrein	34	8	
Kallikrein	34	10	
PSA	30	10	
PSM	347	8	
PSM	347	10	0.0005
PSA	173	9	
PSM	689	9	
PSM	689	11	
Kallikrein	8	10	
PSM	202	8	
PSM	202	9	
PSM	530	8	
PSM	530	10	
PSM	642	8	
PAP	188	11	
PSM	676	9	
PSM	676	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	386	8	
PSM	386	11	
PAP	50	10	
PSA	11	10	
PSM	297	8	
PSM	130	10	
PSM	416	8	
PSM	416	11	
PSM	373	11	
PSA	69	9	
PSA	69	10	
PAP	135	10	
PAP	267	8	
PSM	226	9	
PSM	226	10	
PSM	226	11	
PSM	512	10	
PSM	614	10	0.1900
PSA	175	10	
PSM	52	8	
PSM	52	9	
PSM	52	10	
Kallikrein	226	10	
PSA	222	10	
Kallikrein	25	9	0.0410
PSA	21	9	0.0410
Kallikrein	25	10	
PSA	21	10	
PSM	200	8	
PSM	200	10	
PSM	200	11	
PSM	591	8	
PSM	591	10	
PSM	591	11	
PSM	157	8	
PSM	398	9	0.1700
PSM	398	10	0.0260
PSM	66	8	
PSM	66	10	
PSM	59	8	
PSM	59	9	
PSM	723	8	
PSM	723	11	
PAP	185	9	0.0006
PAP	91	8	
PAP	91	9	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	72	11	
PSA	190	8	
PSM	645	9	
PSM	645	11	
PSM	545	8	
PSM	545	9	
PAP	36	8	
PAP	36	9	
PSM	564	8	
PSM	564	9	
PSM	564	10	
PAP	322	9	0.0002
PAP	322	10	0.0057
PAP	322	11	
PSM	223	10	
PSM	193	11	
PSM	199	9	0.0740
PSM	199	11	
PSM	610	8	
PSM	610	9	0.1800
PSM	514	8	
PSM	514	11	
PAP	282	8	
PSM	304	10	
PSA	166	8	
PAP	193	11	
PAP	173	8	
PAP	173	10	
PSM	491	9	0.4000
PSM	491	10	0.3200
PSM	655	8	
PSM	482	10	0.0044
PSA	66	8	
PSA	66	9	0.0025
PSM	623	11	
PSM	207	9	0.1600
PSM	207	11	
PSM	213	8	
PSM	213	10	
PSM	213	11	
Kallikrein	137	11	
PSA	133	11	
PSM	324	10	
Kallikrein	191	9	
PSA	187	9	
PSA	187	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
Kallikrein	245	10	0.0450
PSA	241	10	0.0450
PSM	219	10	0.0004
PSM	28	10	
PSM	83	8	
PSM	83	11	
PSM	110	10	
PSM	92	10	0.0031
PSM	587	9	
PAP	8	11	
PSM	21	9	
Kallikrein	197	10	
PSA	193	10	
PSM	62	10	
PSM	62	11	
PAP	26	8	
PAP	26	11	
PSM	105	8	
PSM	105	9	
PAP	300	11	
PSM	417	10	
Kallikrein	80	10	
PSM	143	9	
PAP	22	11	
PAP	202	9	
PSA	76	11	
PAP	19	10	
PSM	632	8	
PAP	81	8	
PAP	81	9	0.0002
PAP	81	10	0.0003
PAP	81	11	
PSM	35	8	
PSM	35	10	0.0007
PAP	16	8	
PAP	16	9	
PSM	374	10	
PSM	528	8	
PSM	528	9	0.0006
PSM	528	10	
PAP	191	8	
PSM	679	8	
PSM	679	9	
PSM	679	10	
PSM	679	11	
Kallikrein	139	9	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSA	71	8	
PSM	515	10	
PSM	515	11	
PSM	305	9	0.0006
PAP	21	8	
PSM	34	9	
PSM	34	11	
PSA	70	8	
PSA	70	9	
PSM	428	8	
PSM	4	8	
PSM	4	10	0.0005
Kallikrein	105	8	
PSA	101	8	
PAP	306	9	0.0010
PSM	441	8	
PSM	441	9	
Kallikrein	123	8	
PSA	119	8	
Kallikrein	123	9	
PAP	243	8	
PAP	243	9	0.0760
PAP	243	11	
Kallikrein	178	8	
Kallikrein	178	9	
Kallikrein	178	10	
Kallikrein	178	11	
PSM	116	9	0.0006
PAP	136	9	
PAP	153	11	
PSM	668	8	
Kallikrein	121	10	
PSA	117	10	
Kallikrein	121	11	
PAP	113	9	0.0005
PAP	113	10	0.0005
PSM	469	11	
PAP	148	8	
PAP	148	11	
PAP	238	10	0.0005
PSA	122	10	
PAP	194	10	
PAP	14	10	
PAP	14	11	
PAP	241	10	0.0003
PAP	241	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PAP	244	8	
PAP	244	10	0.0520
Kallikrein	179	8	
Kallikrein	179	9	
Kallikrein	179	10	
Kallikrein	10	8	
PSA	6	8	
PSA	6	9	
PSM	117	8	
PSM	117	11	
PSA	57	8	
PSA	57	10	0.1400
Kallikrein	61	8	
Kallikrein	61	9	
PAP	315	8	0.0014
PAP	315	11	
PSA	4	10	
PSA	4	11	
PSM	268	10	0.0005
PSM	268	11	
PAP	70	9	
PAP	70	10	0.0150
PSA	37	8	
PSM	561	10	
PSM	561	11	
PAP	40	8	0.0003
PSM	473	10	
Kallikrein	54	10	
PSA	50	10	
Kallikrein	54	11	
PSA	50	11	
PSM	26	8	
PAP	263	8	
PAP	263	10	0.0560
PAP	263	11	
PSM	174	8	
Kallikrein	183	9	
PSA	135	9	
PSM	569	9	
Kallikrein	196	11	
PSA	192	11	
Kallikrein	122	9	
PSA	118	9	
Kallikrein	122	10	
PSM	663	8	
PSM	663	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PAP	114	8	
PAP	114	9	
PAP	114	11	
Kallikrein	103	10	
PSA	99	8	
PSA	99	10	0.0070
PAP	117	8	
PSM	451	10	
PSM	216	8	
PSM	195	9	
PSM	195	10	
PSM	195	11	
PSM	519	9	
Kallikrein	181	8	
Kallikrein	181	11	
PSM	665	9	
PSM	665	10	
PSM	665	11	
PSA	177	8	
PSA	177	11	
PSM	336	8	
PSM	336	10	
PSM	638	8	
PSM	638	9	0.0005
PAP	220	8	
PSM	76	9	
PSM	262	11	
PAP	304	8	
PAP	304	11	
PSM	69	9	
PSM	257	8	
PSM	51	9	
PSM	51	10	
PSM	51	11	
Kallikrein	79	11	
PSM	3	9	0.0006
PSM	3	11	
PSM	247	9	
PSM	57	10	
PSM	57	11	
Kallikrein	102	11	
PSM	589	10	
Kallikrein	70	8	
Kallikrein	70	9	
PSM	438	8	
PSM	438	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PAP	34	11	
PSM	480	9	
PAP	237	11	
PAP	240	8	
PAP	240	11	
PSM	560	11	
PAP	317	9	
PAP	317	10	
PSM	621	9	0.0005
PAP	328	10	
PAP	168	10	
PSM	703	9	
PSM	703	11	
PSM	716	8	
PSM	716	9	
PAP	60	8	
PAP	95	9	0.0002
PAP	95	11	
PSM	7	9	
PSM	7	10	
PSM	7	11	
PAP	170	8	
PAP	170	10	0.0004
PAP	170	11	
PSM	542	8	
PSM	542	11	
PSM	557	8	
PSM	557	9	
PSM	557	10	0.0006
PSM	522	10	
PSM	727	9	
PSM	727	10	
PSM	727	11	
PSM	235	8	
PSM	418	9	
PSM	595	11	
PSM	713	11	
PSM	653	10	
PSM	629	9	
PSM	629	11	
PSM	185	9	
PSM	32	8	
PSM	32	11	
PSM	524	8	
PSM	524	11	
PAP	23	10	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	328	10	
PSM	357	9	
PSM	153	9	
PSM	153	11	
PSM	231	10	
PSA	125	9	0.0002
Kallikrein	129	9	
Kallikrein	146	8	
PSA	142	8	
Kallikrein	146	9	
PSA	142	9	
PSM	273	8	
PSM	273	9	0.0001
Kallikrein	240	9	
Kallikrein	240	10	
Kallikrein	240	11	
Kallikrein	233	9	
Kallikrein	233	11	
PSA	229	11	
PSM	484	8	
PSM	484	11	
PSM	682	8	
PSM	682	11	
PSM	368	10	
PSM	368	11	
PSM	315	10	
PSM	594	8	
PAP	157	8	
PSM	685	8	
PSM	685	9	
PAP	345	11	
PSM	331	11	
PSM	706	8	
PSM	270	8	
PSM	270	9	
PSM	270	10	
PSM	270	11	
PAP	49	11	
PSM	296	9	
PAP	57	11	
PAP	134	11	
PSM	678	9	
PSM	678	10	
PSM	678	11	
PAP	5	8	
PSM	468	8	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PAP	147	9	0.0005
PSM	267	8	
PSM	267	11	
PAP	212	8	
PAP	212	10	
PSA	95	9	0.2400
PSA	95	11	
PSM	550	10	0.0004
Kallikrein	99	9	
Kallikrein	99	10	
PSM	568	10	0.0005
PAP	349	8	
PAP	349	9	
PSM	290	10	
PSM	290	11	
PSM	721	9	
PSM	721	10	0.0003
PSA	236	9	
PSA	236	10	0.0079
PSA	236	11	
PSM	502	10	
PSM	694	8	
PAP	224	11	
PAP	278	9	0.0002
PAP	278	11	
PSM	293	8	
PSM	293	10	
Kallikrein	91	8	
Kallikrein	91	11	
PSM	740	11	
PAP	200	9	0.0006
PAP	200	11	
PSM	167	10	
PAP	276	11	
PSM	95	9	
PSM	731	11	
PSM	218	11	
PSM	91	11	
PAP	72	8	
PAP	152	8	
PSM	667	8	
PSM	667	9	
PAP	69	10	
PAP	69	11	
PSM	389	8	
Kallikrein	109	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
Kallikrein	39	10	
Kallikrein	39	11	
PSA	84	9	
PSA	84	11	
PSA	182	9	0.0060
PSA	182	10	
PSA	35	9	0.0021
PSA	35	10	
PSM	578	8	
PSM	578	11	
PSA	87	8	
PSA	87	11	
Kallikrein	72	10	
PAP	101	11	
PAP	2	8	
PAP	2	9	0.1500
PAP	2	10	
PAP	2	11	
PAP	10	9	
PAP	10	11	
PAP	273	8	
PAP	273	9	0.0210
PAP	273	10	0.0053
PSA	43	10	0.0110
Kallikrein	186	10	
PSM	190	10	0.0021
PSM	598	8	
PSM	598	9	0.0024
PSM	598	10	
PSM	598	11	
PSA	105	11	
PAP	163	11	
PSM	363	8	
PSM	363	9	
PSM	580	9	
PSM	255	10	
PSM	210	8	
PSM	210	11	
PSM	320	8	
PSM	445	8	
PSM	511	11	
Kallikrein	24	10	0.0460
PSA	20	10	0.0460
Kallikrein	24	11	
PSA	20	11	
PSM	354	10	0.3700

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	527	8	
PSM	527	9	0.0032
PSM	527	10	
PSM	527	11	
PAP	180	8	
PAP	180	10	0.0005
PSM	440	9	0.0012
PSM	440	10	0.0220
PSA	121	11	
PSM	662	9	
PSM	400	8	
Kallikrein	169	9	
PAP	28	9	0.0490
PAP	28	10	
PSM	181	10	
PSM	414	10	
PAP	111	11	
PSM	463	9	
Kallikrein	89	8	
Kallikrein	89	10	
PAP	115	8	
PAP	115	10	
PSM	312	9	0.0006
PSM	10	8	
PSM	10	10	
PSM	634	10	
PAP	312	8	
PAP	312	9	
PAP	312	11	
PAP	350	8	
PSM	155	9	
PSM	155	10	
PSM	229	8	
PSM	628	8	
PSM	628	10	
PSM	401	11	
PSM	704	8	
PSM	704	10	
PSM	390	11	
PSM	197	8	
PSM	197	9	
PSM	197	11	
PAP	195	9	
PAP	294	10	
PSM	507	8	
PSM	517	8	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	517	9	
PSM	517	11	
PSM	532	8	
Kallikrein	155	11	
PSA	151	11	
PSM	547	10	
Kallikrein	7	11	
PSM	455	9	
Kallikrein	159	9	
Kallikrein	159	11	
PSA	155	11	
PSM	129	11	
PSM	291	9	
PSM	291	10	0.0940
PSM	613	11	
PSM	590	9	0.0006
PSM	590	11	
PSM	142	10	
PSM	631	9	
PAP	15	9	
PAP	15	10	
Kallikrein	175	11	
Kallikrein	104	9	
PSA	100	9	0.0024
PAP	242	9	0.0006
PAP	242	10	0.4900
Kallikrein	170	8	
Kallikrein	110	10	
PAP	13	8	
PAP	13	11	
PSM	472	8	
PSM	472	11	
PSM	492	8	
PSM	492	9	1.0000
PAP	245	9	1.1000
PAP	245	11	
PSA	237	8	
PSA	237	9	0.6800
PSA	237	10	0.2800
PSA	237	11	
PSM	615	9	0.1100
PSM	615	11	
Kallikrein	117	9	0.0039
PSA	113	9	0.0039
PSM	695	11	
PSM	454	10	0.0007

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	45	11	
PSM	317	8	
PSM	317	11	
PAP	106	11	
PAP	369	10	
PSM	431	10	0.0005
PSM	348	9	0.0016
PSM	338	8	
PSM	338	10	
PAP	217	11	
PSA	67	8	
PSA	67	11	
PAP	29	8	0.0017
PAP	29	9	
PSM	626	8	
PSM	626	10	
PSA	7	8	
PSM	554	11	
PSA	58	9	0.0094
Kallikrein	62	8	
PSM	14	10	
PSM	8	8	
PSM	8	9	
PSM	8	10	
PAP	107	10	
PAP	52	8	
Kallikrein	15	10	
PSM	334	8	
PSM	334	10	0.0007
Kallikrein	86	9	
Kallikrein	86	11	
PSA	82	9	0.0002
PSA	82	11	
PSM	415	9	
PAP	190	9	
PSM	404	8	
PSM	404	10	0.0007
PSM	404	11	
PAP	171	9	0.0006
PAP	171	10	0.0007
PAP	112	10	0.0005
PAP	112	11	
PSM	361	10	0.0003
PSM	361	11	
PSM	461	11	
PSA	5	9	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSA	5	10	
PAP	39	9	0.0006
PSM	141	11	
Kallikrein	227	9	
PSA	223	9	
PAP	291	9	
PSM	575	11	
PAP	145	11	
PAP	292	8	
PSM	734	8	
PSM	734	9	
PSM	734	10	
PSM	576	10	
PSM	12	8	
Kallikrein	40	9	
Kallikrein	40	10	
PSA	179	9	
PSA	45	8	
PSM	464	8	
PSM	719	11	
PAP	109	8	
PSM	523	9	
PSM	382	11	
PSA	85	8	
PSA	85	10	
PSM	208	8	
PSM	208	10	
Kallikrein	26	8	
PSA	22	8	
Kallikrein	26	9	
PSA	22	9	
PSM	287	9	
PSM	329	9	
PSM	201	9	
PSM	201	10	
PSM	358	8	
PSA	68	10	
PSA	68	11	
PSM	225	8	
PSM	225	10	
PSM	225	11	
PSA	174	8	
PSA	174	11	
PSM	690	8	
PSM	690	10	0.5400
PSM	690	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSM	27	11	
PAP	30	8	
Kallikrein	138	10	
PSM	115	8	
PSM	115	10	
PSM	592	9	
PSM	592	10	0.0005
PSM	603	8	
PSM	603	10	
PSM	660	11	
PSA	56	9	0.0002
PSA	56	11	
Kallikrein	60	9	
Kallikrein	60	10	
PSA	36	8	
PSA	36	9	
Kallikrein	53	11	
PSA	49	11	
PAP	262	9	0.0019
PAP	262	11	
PSA	134	10	
PSM	154	8	
PSM	154	10	
PSM	154	11	
PSM	627	9	
PSM	627	11	
PAP	293	11	
Kallikrein	92	10	0.0003
PSA	88	10	0.0003
Kallikrein	192	8	
PSA	188	8	
PSA	188	10	0.0003
PAP	38	10	
PSM	394	9	
Kallikrein	246	9	0.0072
PSA	242	9	0.0072
PSM	602	9	0.0390
PSM	602	11	
Kallikrein	47	10	
PAP	226	9	0.0006
PAP	226	11	
Kallikrein	2	8	
PSM	41	9	
PSM	725	9	
PSM	725	11	
Kallikrein	229	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PSA	225	11	
Kallikrein	157	9	
PSA	153	9	
Kallikrein	157	11	
PSA	10	11	
Kallikrein	252	8	
PSA	248	8	
PSM	20	10	0.0026
PAP	25	8	
PAP	25	9	0.0035
Kallikrein	74	8	
PAP	206	9	0.0002
PAP	368	11	
PSM	497	10	
PSA	55	10	0.0004
Kallikrein	59	10	
Kallikrein	59	11	
PSM	607	11	
PSM	700	10	
PSM	692	8	
PSM	692	9	
PSM	692	10	
PSM	179	8	
PSM	179	9	
PAP	310	10	0.0003
PAP	310	11	
PSM	600	8	
PSM	600	9	
PSM	600	11	
PSM	277	8	
PSM	277	10	
PAP	214	8	
PSM	709	10	
PSM	300	9	0.0006
PSA	97	9	
PSA	97	10	
PAP	210	10	
PSM	566	8	
PSM	113	10	0.0005
PSM	234	9	
PAP	319	8	
PAP	325	8	
PAP	247	9	0.0006
PAP	247	11	
PSM	205	9	0.0006
PSM	205	11	

Table XVI
Prostate A03 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*0301
PAP	84	8	
PAP	84	9	
PAP	103	9	
PAP	155	9	
PAP	155	10	
PSM	228	8	
PSM	228	9	
Kallikrein	188	8	
PSM	471	9	0.0600
PSM	625	9	
PSM	625	11	
PSM	537	9	
PSM	537	10	
Kallikrein	243	8	
PSA	239	8	
Kallikrein	243	9	0.0006
PSA	239	9	0.0006
PSM	733	9	
PSM	733	10	
PSM	733	11	
PSM	371	8	
PSM	176	10	
PSM	176	11	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PSA	59	8	
PSA	13	8	
PAP	3	8	
PSM	392	9	
PSM	608	10	
PSM	608	11	
PSM	452	9	
PSM	232	9	0.0051
PSM	232	11	
PSM	674	11	
PSM	220	9	
PSM	264	9	
PSM	701	9	
Kallikrein	199	8	
PSA	195	8	
PSM	84	11	
PSM	711	8	
Kallikrein	235	9	
Kallikrein	235	11	
PSA	231	9	0.0013
PSA	231	11	
PSM	274	8	
PSM	588	11	
PAP	311	9	0.0550
PSM	531	9	0.2700
PAP	227	8	0.0039
PAP	227	10	
PAP	189	10	
PSM	49	8	
PSM	49	11	
PAP	274	8	0.0700
PAP	274	9	1.2000
PSM	11	9	
PSA	44	9	
PSM	286	10	
PSM	635	11	
Kallikrein	17	8	
PSM	393	8	
PSM	601	10	0.0210
Kallikrein	41	9	
Kallikrein	241	8	
Kallikrein	241	9	
Kallikrein	241	10	
Kallikrein	241	11	
Kallikrein	198	9	
PSA	194	9	0.0015

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
Kallikrein	234	10	
PSA	230	10	
PSA	180	8	
PSA	180	11	
Kallikrein	184	8	
PSM	196	9	
PSM	196	10	0.0490
PAP	347	9	0.0006
Kallikrein	14	11	
PSM	466	10	
PSM	710	9	0.0002
PSM	301	8	
PSM	596	10	
PSM	596	11	
PSM	465	11	
PSA	111	11	
PSM	652	11	
PSM	520	8	
PSM	184	10	
PAP	186	8	
PSM	714	10	0.0002
PAP	201	8	
PAP	201	10	
PSM	173	9	
Kallikrein	182	10	
PSM	191	9	
PSA	98	8	0.0001
PSA	98	11	
PSM	9	8	
PSM	9	9	
PSM	9	11	
PSM	630	8	
Kallikrein	116	10	
PSA	112	10	
PSM	453	8	
PSM	453	11	
PSM	316	9	0.0003
PSM	106	8	
PAP	51	9	0.0001
Kallikrein	85	10	
PSA	81	10	
PSA	178	10	0.0011
PSM	114	9	0.0010
PSM	114	11	
PAP	301	10	
PSM	48	8	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PSM	48	9	
PSM	285	11	
PAP	371	8	
PSM	183	8	
PSM	183	11	
PAP	150	10	
Kallikrein	115	11	
Kallikrein	84	11	
PSA	80	11	
PAP	229	8	
PSM	102	11	
PAP	176	9	
PAP	176	10	
PSM	505	10	
PSM	171	9	
PSM	171	11	
PSM	486	9	
PSM	489	11	
PSM	641	9	0.0002
PAP	266	8	
PSM	397	10	
PSM	397	11	
PSM	109	11	
PAP	166	8	
PAP	80	8	
PAP	80	9	
PAP	80	10	
PAP	80	11	
PSM	64	8	
PSM	64	9	
PAP	34	10	0.0037
PAP	34	11	
PAP	237	11	
PAP	240	8	
PAP	240	11	
PAP	317	9	
PAP	328	10	
PSM	68	8	
PSM	437	9	
PSM	716	8	
PAP	95	9	0.0002
PAP	95	11	
PSM	7	10	
PSM	7	11	
PAP	170	10	0.0140
PAP	170	11	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PSM	542	8	
PSM	542	11	
PSM	557	8	
PSM	557	10	0.0002
PSM	235	8	
PSM	595	11	
PSM	713	11	
PSM	653	10	
PSM	629	9	
PSM	185	9	
PSM	524	11	
PAP	23	11	
PAP	203	8	
PSM	103	10	
PSM	103	11	
PSM	402	10	
PSM	675	10	
PSM	61	11	
PSM	37	8	
PAP	18	11	
PAP	20	9	0.0004
PAP	92	8	
PSA	106	10	
PSM	73	10	0.0036
PSM	646	10	0.0007
PSM	506	9	
PSM	546	8	
PSM	546	11	
PSM	337	9	
PSM	337	11	
PSM	639	11	
PSM	333	9	
PSM	333	11	
PAP	37	8	
PAP	37	11	
PSA	12	9	0.0350
PSM	391	10	
PSM	263	10	
PSM	221	8	
PSM	364	8	
Kallikrein	16	9	
PAP	346	10	
PSM	172	8	
PSM	172	10	
PSM	265	8	
PSM	487	8	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PSM	36	9	0.0014
PSM	332	10	
PSM	310	11	
PAP	260	11	
Kallikrein	27	8	
PSA	23	8	
PSM	529	8	
PSM	529	9	
PSM	529	11	
PAP	248	8	
PAP	248	10	
PAP	204	11	
PSM	104	9	
PSM	104	10	
PAP	305	10	
PSM	680	8	
PSM	680	9	0.0280
PSM	680	10	
PSM	288	8	
PAP	295	9	
PAP	74	11	
PSM	168	9	0.0002
PSM	518	10	
PSM	335	9	
PSM	335	11	
PSM	311	10	0.1400
PSA	226	10	
Kallikrein	158	10	
PSM	430	11	
PSM	85	10	
PSM	403	9	
PSM	403	11	
PSM	360	11	
PSM	224	11	
PAP	261	10	
Kallikrein	49	8	
PAP	198	11	
PSM	345	10	
Kallikrein	177	10	
PAP	314	9	0.5300
PSM	573	8	
PAP	270	11	
PSM	475	8	
PSM	56	11	
Kallikrein	94	8	0.0006
PSA	90	8	0.0006

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
Kallikrein	34	10	
PSM	347	8	
PSM	347	10	0.0002
PSM	689	9	
PSM	689	11	
PSM	202	9	
PSM	530	8	
PSM	530	10	
PSM	642	8	
PAP	188	11	
PSM	676	9	
PSM	386	11	
PAP	50	10	
PSA	11	10	
PSM	297	8	
PSA	69	10	
PAP	135	10	
PSM	226	9	
PSM	450	11	
PSM	194	11	
PSM	614	10	0.1100
PSA	175	10	
PSM	52	8	
Kallikrein	25	9	0.0190
PSA	21	9	0.0190
Kallikrein	25	10	
PSA	21	10	
PSM	200	8	
PSM	200	11	
PSM	591	8	
PSM	591	10	
PSM	398	9	0.0087
PSM	398	10	0.0006
PSM	66	10	
PSM	59	8	
PSM	723	8	
PSM	723	11	
PAP	185	9	0.0004
PAP	91	8	
PAP	91	9	
PSM	72	11	
PSA	190	8	
PSM	645	11	
PSM	545	8	
PSM	545	9	
PAP	36	8	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PAP	36	9	
PSM	564	8	
PSM	564	9	
PSM	564	10	
PAP	322	9	0.0002
PAP	322	10	0.0890
PAP	322	11	
PSM	199	9	1.0000
PSM	610	8	
PSM	610	9	0.1200
PAP	282	8	
PSA	166	8	
PSM	215	9	
PSM	637	9	
Kallikrein	69	9	
Kallikrein	69	10	
PSM	539	11	
PAP	173	8	
PAP	173	10	
PSM	491	9	2.1000
PSM	491	10	0.0810
PSM	655	8	
PSM	482	10	0.0210
PSA	66	8	
PSA	66	9	0.0014
PSM	207	9	0.1200
PSM	213	11	
PSA	187	11	
Kallikrein	245	10	0.0450
PSA	241	10	0.0450
PSM	219	10	0.0002
PSM	110	10	
PSM	92	10	0.0007
Kallikrein	197	10	
PSA	193	10	
PSM	62	10	
PSM	62	11	
PAP	26	8	
PAP	26	11	
PSM	105	8	
PSM	105	9	
PAP	300	11	
Kallikrein	80	10	
PSM	143	9	
PAP	202	9	
PAP	19	10	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PAP	81	8	
PAP	81	9	0.0002
PAP	81	10	0.0002
PAP	81	11	
PSM	35	10	0.3700
PSM	528	9	0.0002
PSM	528	10	
PAP	191	8	
PSM	679	9	
PSM	679	10	
PSM	679	11	
PSA	71	8	
PAP	21	8	
PSM	34	11	
PSA	70	9	
Kallikrein	105	8	
PSA	101	8	
PAP	306	9	0.0002
PSM	441	9	
Kallikrein	123	9	
PAP	243	8	
PAP	243	9	0.2000
PAP	243	11	
Kallikrein	178	9	
Kallikrein	178	11	
PSM	116	9	0.0003
PAP	136	9	
PAP	153	11	
Kallikrein	121	11	
PSM	469	11	
PAP	93	11	
PAP	148	8	
PAP	238	10	0.0004
PAP	241	10	0.0002
PAP	241	11	
PAP	244	8	
PAP	244	10	0.0370
Kallikrein	179	8	
Kallikrein	179	10	
PSM	117	8	
PSM	117	11	
PSA	57	8	
PSA	57	10	0.0830
Kallikrein	61	8	
Kallikrein	61	9	
PAP	315	8	0.0100

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PAP	315	11	
PSM	268	10	0.0002
PAP	70	9	
PAP	70	10	0.0024
PSM	561	11	
PAP	40	8	0.0002
PSM	473	10	
PAP	263	8	
PAP	263	10	0.1200
PAP	263	11	
PSM	174	8	
Kallikrein	183	9	
Kallikrein	196	11	
PSA	192	11	
Kallikrein	122	10	
PSM	663	11	
PSM	664	10	
Kallikrein	103	10	
PSA	99	10	0.0110
PSM	451	10	
PSM	216	8	
PSM	195	10	
PSM	195	11	
PSM	519	9	
Kallikrein	181	8	
Kallikrein	181	11	
PSM	665	9	
PSA	177	8	
PSA	177	11	
PSM	336	8	
PSM	336	10	
PSM	638	8	
PSM	262	11	
PAP	304	11	
PSM	51	9	
Kallikrein	79	11	
PSM	247	9	
PSM	57	10	
Kallikrein	102	11	
PSM	589	10	
Kallikrein	70	8	
Kallikrein	70	9	
PSM	438	8	
PSM	231	10	
PSA	125	9	0.0002
Kallikrein	129	9	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein		Position	No. of Amino Acids	A*1101
Kallikrein	PALGTTCY	146	8	
PSA	PALGTTCY	142	8	
PSM	PANEYAYR	273	8	
PSM	PANEYAYRR	273	9	0.0002
Kallikrein	PAVYTKVVH	240	9	
Kallikrein	PAVYTKVVHY	240	10	
Kallikrein	PAVYTKVVHYR	240	11	
Kallikrein	PCALPEKPAVY	233	11	
PSA	PCALPERPSLY	229	11	
PSM	PDEGFEGK	484	8	
PSM	PDEGFEGKSLY	484	11	
PSM	PDRPFYRH	682	8	
PSM	PDRPFYRHVIY	682	11	
PSM	PDRYVILGGH	368	10	
PSM	PDRYVILGGHR	368	11	
PSM	PDSSWRGSLK	315	10	
PSM	PFYRHVIY	685	8	
PAP	PGCSPSCPLER	345	11	
PSM	PGFTGNFSTQK	331	11	
PSM	PGYPANEY	270	8	
PSM	PGYPANEYAY	270	10	
PSM	PGYPANEYAYR	270	11	
PAP	PIDTFPTDPIK	49	11	
PSM	PIGYYDAQK	296	9	
PAP	PILLWQPIPVH	134	11	
PSM	PLGLPDRPFY	678	10	
PSM	PLGLPDRPFYR	678	11	
PSM	PLMYSLVH	468	8	
PAP	PLSEDQLLY	147	9	0.0001
PSM	PLTPGYPANEY	267	11	
PAP	PLYCESVH	212	8	
PSA	PLYDMSLLK	95	9	0.0370
PSA	PLYDMSLLKNR	95	11	
PSM	PLYHSVYETY	550	10	0.0002
Kallikrein	PLYNMSLLK	99	9	
Kallikrein	PLYNMSLLKH	99	10	
PSM	PNKTHPNY	120	8	
PSM	PSIPVHPIGY	290	10	
PSM	PSIPVHPIGYY	290	11	
PSM	PSKAWGEVK	721	9	
PSM	PSKAWGEVKR	721	10	0.0002
PSA	PSLYTKVVH	236	9	
PSA	PSLYTKVVHY	236	10	0.0003
PSA	PSLYTKVVHYR	236	11	
PSM	PSPEFSGMPR	502	10	
PAP	PSWATEDTMTK	224	11	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PAP	278	9	0.0002
PSM	293	8	
Kallikrein	91	8	
Kallikrein	91	11	
PAP	200	9	0.0008
PAP	200	11	
PSM	167	10	
PAP	276	11	
PSM	218	11	
PSM	91	11	
PAP	72	8	
PAP	152	8	
PAP	69	10	
PAP	69	11	
PSM	389	8	
Kallikrein	109	11	
Kallikrein	39	11	
PSA	84	11	
PSA	182	9	0.0140
PSA	35	9	0.0018
PSA	87	8	
PSA	87	11	
PAP	101	11	
PAP	2	9	0.1200
PAP	273	8	
PAP	273	9	0.0600
PAP	273	10	0.0250
PSA	43	10	0.0310
PSM	190	10	0.0002
PSM	598	8	
PSM	598	9	0.0190
PSM	598	10	
PSA	105	11	
PAP	163	11	
PSM	363	8	
PSM	363	9	
PSM	320	8	
Kallikrein	24	10	0.0670
PSA	20	10	0.0670
Kallikrein	24	11	
PSA	20	11	
PSM	354	10	0.4300
PSM	527	8	
PSM	527	10	
PSM	527	11	
PSM	440	10	0.0005

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PAP	332	9	0.0002
PSA	64	10	
PSA	64	11	
PSM	400	8	
Kallikrein	169	9	
PAP	28	9	0.1100
PSM	181	10	
PSM	463	9	
Kallikrein	89	10	
PSM	312	9	0.0012
PSM	10	8	
PSM	10	10	
PAP	312	8	
PAP	312	11	
PSM	628	10	
PSM	401	11	
PSM	390	11	
PSM	197	8	
PSM	197	9	
PSM	197	11	
PAP	294	10	
PSM	507	8	
PSM	517	11	
PSM	532	8	
PSM	547	10	
PSM	455	9	
Kallikrein	159	9	
Kallikrein	159	11	
PSA	155	11	
PSM	291	9	
PSM	291	10	1.4000
PSM	613	11	
PSM	590	9	0.0220
PSM	590	11	
PSM	142	10	
Kallikrein	104	9	
PSA	100	9	0.0470
PAP	242	9	0.0002
PAP	242	10	2.3000
Kallikrein	170	8	
Kallikrein	110	10	
PSM	472	8	
PSM	472	11	
PSM	492	8	
PSM	492	9	2.0000
PAP	245	9	0.8000

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PAP	245	11	
PSA	237	8	
PSA	237	9	0.0140
PSA	237	10	0.2300
PSA	237	11	
PSM	615	9	0.0720
PSM	615	11	
Kallikrein	180	9	
PSA	176	9	
PSM	46	10	
PSM	46	11	
Kallikrein	117	9	1.2000
PSA	113	9	1.2000
PSM	454	10	0.0910
PSM	45	11	
PSM	317	8	
PSM	317	11	
PAP	369	10	
PSM	431	10	0.0016
PSM	348	9	0.0083
PSM	338	8	
PSM	338	10	
PSA	67	8	
PAP	29	8	0.0061
PSM	554	11	
PSA	58	9	0.0140
Kallikrein	62	8	
PSM	8	9	
PSM	8	10	
PAP	52	8	
Kallikrein	15	10	
PSM	334	8	
PSM	334	10	0.0002
Kallikrein	86	9	
PSA	82	9	0.0002
PAP	190	9	
PSM	404	8	
PSM	404	10	0.0002
PSM	404	11	
PAP	171	9	0.0078
PAP	171	10	0.0001
PSM	361	10	0.0002
PSM	361	11	
PSM	461	11	
PAP	39	9	0.0002
PSM	349	8	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PSM	50	10	
PSM	543	10	
PSM	543	11	
PSM	141	11	
PAP	145	11	
PSM	12	8	
Kallikrein	40	10	
PSA	179	9	
PSA	45	8	
PSM	464	8	
PSM	719	11	
PSA	85	10	
PSM	208	8	
Kallikrein	26	8	
PSA	22	8	
Kallikrein	26	9	
PSA	22	9	
PSM	287	9	
PSM	201	10	
PSA	68	11	
PSM	225	10	
PSA	174	11	
PSM	690	8	
PSM	690	10	0.7900
PSM	690	11	
PSM	115	8	
PSM	115	10	
PSM	592	9	
PSM	603	8	
PSM	603	10	
PSA	56	9	0.0005
PSA	56	11	
Kallikrein	60	9	
Kallikrein	60	10	
PSA	36	8	
PAP	262	9	0.0030
PAP	262	11	
PAP	264	9	
PAP	264	10	
PSM	177	11	
PSM	627	11	
PAP	293	11	
Kallikrein	92	10	0.0015
PSA	88	10	0.0015
PSA	188	10	0.0120
PAP	38	10	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
Kallikrein	246	9	0.0930
PSA	242	9	0.0930
PSM	602	9	0.0660
PSM	602	11	
Kallikrein	47	10	
PAP	226	9	0.0002
PAP	226	11	
PSM	725	9	
Kallikrein	229	11	
PSA	225	11	
Kallikrein	157	11	
PSA	10	11	
PAP	25	9	0.0150
PSM	246	10	
PAP	206	9	0.0002
PAP	368	11	
PSA	55	10	0.0001
Kallikrein	59	10	
Kallikrein	59	11	
PSM	607	11	
PSM	700	10	
PSM	692	8	
PSM	692	9	
PSM	179	9	
PAP	310	10	0.0002
PSM	600	8	
PSM	600	11	
PSM	709	10	
PSM	300	9	0.0002
PSA	97	9	
PAP	210	10	
PSM	566	8	
PSM	113	10	0.0016
PSM	234	9	
PAP	325	8	
PAP	247	9	0.0002
PAP	247	11	
PSM	205	9	0.0002
PSM	205	11	
PAP	84	8	
PAP	103	9	
PAP	155	9	
PSM	75	8	
PAP	303	8	
Kallikrein	101	8	
PSM	356	8	

Table XVII
Prostate A11 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*1101
PSM	471	9	0.5400
PSM	537	9	
Kallikrein	243	8	
PSA	239	8	
Kallikrein	243	9	0.0580
PSA	239	9	0.0580
PSM	371	8	

Table XVIII
Prostate A24 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	674	8	
PSM	60	11	
PSM	736	11	
PAP	116	8	
PAP	116	9	0.0150
PSM	724	9	
PSM	448	9	0.0190
Kallikrein	187	9	
Kallikrein	187	11	
Kallikrein	152	9	0.1700
PSA	148	9	0.1700
PSM	652	8	
PSM	652	10	
PSM	520	9	
PSM	520	11	
PSM	184	11	
PAP	186	9	0.0002
PSM	191	10	
PSA	98	9	0.0001
PSA	98	10	
PSM	102	9	
PSM	425	10	
Kallikrein	164	8	
PSA	160	8	
Kallikrein	194	8	
Kallikrein	194	9	
PSM	505	8	
PSM	505	11	
PSM	621	9	0.0010
PSM	433	9	
PSM	433	10	
PSM	276	8	
PAP	83	10	0.0067
PAP	83	11	
PSM	185	10	
PSM	32	8	
PSM	32	10	0.0026
PSM	32	11	
PAP	23	9	0.0017
Kallikrein	195	8	
PSA	191	8	
PAP	24	8	
PSM	565	10	1.1000
PSM	487	11	
PSM	31	8	
PSM	31	9	0.0190

Table XVIII
Prostate A24 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	31	11	
PAP	66	8	
PSM	36	8	
PAP	17	8	
PAP	17	9	0.0016
PAP	17	10	0.0007
PAP	74	8	
PSM	508	8	
PSM	582	10	0.0002
Kallikrein	46	9	
Kallikrein	28	11	
PSA	24	11	
Kallikrein	156	10	0.0001
PSA	152	10	0.0001
Kallikrein	156	11	
PSA	152	11	
PSM	409	8	
PSM	409	9	
PSM	409	10	0.0540
PSM	150	8	
PSM	298	8	
PSM	298	9	
PAP	270	8	
PAP	78	8	
Kallikrein	248	10	0.0550
PSA	244	10	0.0550
PAP	131	8	
PAP	131	11	
PAP	205	9	0.0024
PSM	708	8	
PSM	355	8	
PSM	72	9	
PSA	190	9	0.0310
PSM	645	9	
PSM	564	11	
PSM	606	9	12.0000
PSM	699	10	
PSM	417	10	
PAP	22	10	0.0045
PSA	76	11	
PAP	19	8	
PAP	123	9	0.0033
PAP	123	10	0.0140
PSM	632	8	
PSM	632	11	
PSM	668	9	

Table XVIII
Prostate A24 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	668	9	0.0075
PAP	113	8	
PAP	113	11	
PSM	469	9	
PAP	213	9	0.4400
PAP	213	11	
PSA	96	11	0.1200
PAP	318	9	2.5000
PSM	551	11	
PAP	154	11	
PSM	74	10	0.2300
PSM	227	9	0.4400
PSA	238	11	
PSM	669	8	
PSM	669	11	
PSM	663	8	
PSM	663	9	
Kallikrein	1	8	
Kallikrein	1	10	
PSM	470	8	
PSM	89	8	
PSM	336	11	
PSM	638	9	0.0001
PSM	76	8	
PSM	57	9	
Kallikrein	102	10	
PSM	178	8	
PSM	178	9	0.7700
PSM	178	11	
PSM	459	11	
PSM	594	11	
PAP	157	8	
PAP	157	11	
Kallikrein	37	11	
PAP	309	10	0.0240
PAP	183	9	0.1100
PSM	326	8	
PAP	297	10	0.0001
PAP	297	11	
PSA	54	10	0.0007
Kallikrein	58	10	
PAP	355	10	0.0037
PAP	163	10	0.0001
PSM	662	9	
PSM	662	10	
PSM	19	8	

Table XVIII
Prostate A24 Motif Peptides with Binding Data

Protein	Position	No. of Amino Acids	A*2401
PSM	19	10	
PSM	536	11	
PSM	401	10	
PSM	704	9	
PSM	704	10	
PSA	91	11	
Kallikrein	95	11	
PAP	225	11	
PSM	420	9	
PSM	420	10	
Kallikrein	228	9	
PSA	224	9	0.0001
PAP	62	9	0.0013
PSM	496	11	
PAP	96	9	0.2600
PSM	241	8	
PSM	118	11	
PAP	231	8	
PAP	231	11	
PSA	9	8	
PSA	9	9	0.1100
PSA	9	10	0.3600
PSM	558	8	
PSM	624	9	
PSM	624	10	3.2000
PSM	584	8	
PSM	584	10	
PSM	523	8	
PSA	2	9	2.1000
PSA	2	10	0.0062
PSA	85	8	
PAP	41	10	0.0005
PSA	134	11	
Kallikrein	73	8	
Kallikrein	73	9	
PSM	555	11	
Kallikrein	242	11	
PSM	175	11	
PAP	319	8	
PSM	299	8	

Table XIX
Prostate DR Supermotif Peptides

Protein	Position
PAP	1
Kallikrein	1
PSA	1
Kallikrein	2
PSA	2
PSA	3
PAP	124
PSA	16
PAP	6
PAP	14
PSM	611
PSM	287
PSM	426
PAP	360
PSA	198
PSA	63
PAP	35
PAP	302
Kallikrein	12
PSA	17
PAP	7
Kallikrein	188
Kallikrein	157
PSA	153
PSM	289
PSA	134
Kallikrein	20
PSA	183
PAP	218
Kallikrein	222
PSA	218
PAP	164
PSM	469
PSM	488
PSM	523
PSA	174
Kallikrein	6
PSM	570
PSM	669
PSM	686
PAP	30
PAP	113
PSM	456
PAP	293
Kallikrein	166

Table XIX
Prostate DR Supermotif Peptides

Protein	Position
PSM	105
PSM	192
PSM	447
PSM	719
PSM	525
PSM	279
PAP	359
PAP	26
PAP	70
PAP	21
PSA	6
PAP	167
PSM	164
PSM	549
PSM	642
PSM	394
PSM	175
PSM	268
PSM	33
PSM	253
PSA	213
Kallikrein	217
PAP	263
PSM	493
PSM	209
PSM	585
PSM	138
PSM	259
PSM	214
PSM	333
PSA	214
Kallikrein	218
PAP	364
PAP	202
Kallikrein	90
PSA	86
PSA	45
PSM	449
PSM	227
PSA	51
Kallikrein	55
PAP	131
PSM	248
PSA	118
Kallikrein	122
PSM	399

Table XIX
Prostate DR Supermotif Peptides

Protein	Position
PAP	340
PAP	102
Kallikrein	81
PSA	97
Kallikrein	101
PSA	55
Kallikrein	59
PSA	77
PSM	556
PSM	115
PAP	53
PSM	300
PSM	73
PAP	138
PAP	280
Kallikrein	229
PSA	225
PSM	614
PSM	62
PSM	410
PSM	75
PSM	226
Kallikrein	242
PAP	258
PSM	344
PSM	574
PSM	113
PSM	65
PAP	303
PSM	309
PAP	25
PSM	41
PSM	38
Kallikrein	179
PAP	184
PSA	175
PAP	286
PAP	24
PAP	156
PSM	671
PSA	120
Kallikrein	124
PAP	310
PSM	292
PAP	226
PSA	170

Table XIX
Prostate DR Supermotif Peptides

Protein	Position
Kallikrein	174
PSM	653
Kallikrein	226
PSA	222
PAP	238
PSM	664
PAP	241
PAP	197
PAP	244
PSM	177
PSM	572
PSM	512
PAP	117
Kallikrein	106
PSA	102
PAP	120
Kallikrein	4
PSM	473
PAP	97
PAP	223
PAP	307
Kallikrein	223
PSA	219
Kallikrein	105
PAP	136
PSM	592
PSM	143
PSM	462
PSM	234
Kallikrein	236
PSA	232
Kallikrein	165
PAP	129
PSA	96
Kallikrein	100
PAP	137
PAP	143
PSA	167
PAP	8
PAP	344
PAP	368
PSM	622
PSM	169
PSA	188
Kallikrein	171

Table XIX
Prostate DR Supermotif Peptides

Protein	Position
PSM	329
PAP	342
PAP	262
PSM	734
PSM	100
Kallikrein	75
PAP	104
PSA	57
Kallikrein	61
PSM	676
PSM	381
PSM	583
PSM	691
Kallikrein	253
PSA	249
PSM	530
PSM	20
PSA	238
PSM	733
PAP	50
Kallikrein	92
PSM	158
Kallikrein	192
PSA	117
Kallikrein	121
Kallikrein	10
PAP	210
Kallikrein	178
PAP	16
PSM	659
PSA	34
PSA	22
Kallikrein	26
PSM	442
PAP	109
PSM	434
PSM	110
PSA	70
PSM	629
PSA	10
PSM	383
PSA	132
Kallikrein	136
Kallikrein	196
Kallikrein	18
PSM	337

Table XIX
Prostate DR Supermotif Peptides

Protein	Position
PSM	418
PSM	464
PSA	8
PSM	546
PSM	356
PSM	144
PAP	148
PSM	627
PSM	737
PSM	579
Kallikrein	43
PSM	450
PAP	330
PSM	587
PSA	88
PSM	297
PSA	71
PSM	639
Kallikrein	141
PSM	663
PSA	137
Kallikrein	21
PSM	161
PSM	157
PAP	132
PSA	11
PSA	4
Kallikrein	138
Kallikrein	5
PSM	103
PSM	5
PAP	135
PAP	141
PSM	603
PSM	712
PAP	213
PSM	569
PSM	154
PSM	497
PAP	283
PAP	306
PAP	343
PSM	690
Kallikrein	252
PSA	248

Table XXa
Prostate DR 3a Submotif Peptides

Protein	Position
PAP	124
PSM	669
PSM	186
PAP	331
PSM	405
PAP	167
PSM	394
PAP	263
PAP	298
PAP	364
PSM	227
PSM	700
Kallikrein	81
Kallikrein	111
PSA	77
PAP	53
PSM	131
PAP	325
PSM	65
Kallikrein	179
PSA	175
PAP	24
PAP	318
PSM	4
PAP	97
PSM	441
PSM	462
PSM	366
PSM	583
PAP	172
PAP	148
PSM	627
PSM	450
PSM	663
Kallikrein	160
PSA	156
PSM	103
PAP	213
PSM	130
PAP	92

Table XXb
Prostate DR 3b Submotif Peptides

Protein		Position
PSM	IQSQWKEFG	96
PSM	FDIESKVDP	713
PSM	YSISMKHPQ	612
PSM	INCSGKIVI	194
PAP	YCESVHNFT	214
PSM	LERDMKINC	188
PSM	YAPSSHNKY	692
PSM	VIGTLRGAV	358
PAP	IMYSAHDTT	284
PAP	LQMEQHYEL	73
PSM	FLDELKAEN	61
PSM	AWGEVKRQI	724
PAP	LNESYKHEQ	93
PAP	LAKELKFVT	31
PSM	LPFDCRDYA	593
PSA	VCAQVHPQK	179
PSM	AVATARRPR	11
PAP	MTTNSHQGT	373
PSM	AEENSRLLQ	435
PSM	LTKELKSPD	477

TABLE XXI. Population coverage with combined HLA Supertypes

HLA-SUPERTYPES	PHENOTYPIC FREQUENCY					
	Caucasian	North American Black	Japanese	Chinese	Hispanic	Average
a. Individual Supertypes						
A2	45.8	39.0	42.4	45.9	43.0	43.2
A3	37.5	42.1	45.8	52.7	43.1	44.2
B7	43.2	55.1	57.1	43.0	49.3	49.5
A1	47.1	16.1	21.8	14.7	26.3	25.2
A24	23.9	38.9	58.6	40.1	38.3	40.0
B44	43.0	21.2	42.9	39.1	39.0	37.0
B27	28.4	26.1	13.3	13.9	35.3	23.4
B62	12.6	4.8	36.5	25.4	11.1	18.1
B58	10.0	25.1	1.6	9.0	5.9	10.3
b. Combined Supertypes						
A2, A3, B7	84.3	86.8	89.5	89.8	86.8	87.4
A2, A3, B7, A24, B44, A1	99.5	98.1	100.0	99.5	99.4	99.3
A2, A3, B7, A24, B44, A1, B27, B62, B58	99.9	99.6	100.0	99.8	99.9	99.8

Table XXII. Prostate Antigen Peptides

Antigen Binding affinity ≤ 200nM	Sequence
PSA.117	LMLLRLSEPA
PSA.118	MLLRLSEPAEL
PSA.118	MLLRLSEPA
PSA.143	ALGTTCYA
PSA.161	FLTPKKLQCV
PSA.166	KLQCVDLHV
PAP.6	LLLARAASLSL
PAP.21	LLFFWLDRSV
PAP.30	VLAKELKFV
PAP.92	FLNESYKHEQV
PAP.112	TLMSAMTNL
PAP.135	ILLWQPIPV
PAP.284	IMYSAHDTTV
PAP.299	ALDVYNGLL
PSM.26	LVLAGGFFL
PSM.27	VLAGGFFLL
PSM.168	GMPEGDLVYV
PSM.288	GLPSIPVHPI
PSM.441	LLQERGVAYI
PSM.469	LMYSLVHNL
PSM.662	RMMNDQLMFL
PSM.663	MMNDQLMFL
PSM.667	QLMFLERAFI
PSM.711	ALFDIESKV
HuK2.165	FLRPRSLQCV
HuK2.175	SLHLLSNDMCA

Binding affinity >200nM	Sequence
PSM.4	LLHETDSAV
PSM.25	ALVLAGGFFL
PSM.427	GLLGSTEWA
PSM.514	KLGSGNDFEV

Table XXIIIA A2 supermotif cross-reactive binding data

Peptide	AA	Sequence	Source	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	A2 Cross-Reactivity
20.0044	9	LLLARAASL	PAP.6	208	13	29	425	--	4
63.0136	11	LLLARAASLSL	PAP.6	8.1	3.1	5.3	80	143	5
60.0201	9	LLLARAASV	PAP.6.V9	18	215	6.7	95	--	4
20.0203	10	LLARAASLSL	PAP.7	500	5.2	63	9250	5714	3
63.0031	10	LLARAASLSV	PAP.7.V10	109	10	21	378	727	4
63.0137	11	AASLSLGFLFL	PAP.11	227	29	53	95	--	4
1419.51	10	SLSLGFLFLL	PAP.13	40	13	403	21	8560	4
1419.52	10	SLSLGFLFLV	PAP.13.V10	1.8	3.9	17	42	355	5
1419.50	9	SLSLGFLFV	PAP.13.V9	77	25	21	93	--	4
60.0203	9	FLFLLFFWV	PAP.18.V9	42	307	625	308	90	4
63.0138	11	FLLFFWLDRSV	PAP.20	14	17	2.8	285	364	5
1097.09	10	LLFFWLDRSV	PAP.21	28	0.60	1.6	231	--	4
1418.23	10	LTFFWLDRSV	PAP.21.T2	118	11	9.6	43	16	5
63.0139	11	LLFFWLDRSVL	PAP.21	65	2.9	2.7	822	4444	3
63.0033	10	SLLAKELKFV	PAP.29.L2	64	5.7	3.8	38	6667	4
1097.171	9	VLAKELKFV	PAP.30	96	3.6	6.7	168	--	4
63.0142	11	VLAKELKFVTL	PAP.30	6.9	8.1	21	25	--	4
63.0034	10	VLAKELKFVV	PAP.30.V10	31	12	189	86	2286	4
1419.55	11	FLNESYKHEQV	PAP.92	29	1.4	5.6	381	6154	4
1177.01	9	TLMSAMTNL	PAP.112	43	0.80	2.9	285	296	5
20.0312	10	TLMSAMTNLA	PAP.112	385	3.6	37	3700	6667	3
63.0037	10	TLMSAMTNLV	PAP.112.V10	63	3.9	12	43	242	5
1419.56	9	TLMSAMTNV	PAP.112.V9	10	2.4	3.6	54	62	5
1419.58	10	LLALFPPEGV	PAP.120.L2	5.0	0.70	1.6	148	163	5
1419.59	10	LVALFPPEGV	PAP.120.V2	156	17	4.8	463	28	5
1419.6	10	ALFPPEGVSI	PAP.122	278	11	133	2643	--	3
1419.61	10	ALFPPEGVSV	PAP.122.V10	15	1.0	18	119	4444	4
63.0041	10	GVSIWNPILV	PAP.128.V10	250	94	23	451	2286	4

-- indicates binding affinity >10,000nM.

Table XXIIIA A2 supermotif cross-reactive binding data

Peptide	AA	Sequence	Source	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	A2 Cross-Reactivity
60.0207	9	GVSIWNPIV	PAP.128.V9	455	269	909	308	--	3
63.0042	10	PLLLWQPIPV	PAP.134.L2	238	47	19	336	3333	4
1044.04	9	ILLWQPIPV	PAP.135	3.3	39	1.8	71	1702	4
1418.25	9	ITLWQPIPV	PAP.135.T2	34	1720	6.2	26	32	4
1419.69	10	LLWQPIPVHV	PAP.136.V10	25	1.8	17	287	60	5
1166.11	10	GLHGQDLFGI	PAP.196	26	0.90	2.5	315	--	4
1419.62	10	GLHGQDLFGV	PAP.196.V10	12	2.3	3.1	18	--	4
63.0048	10	KLRELSELSV	PAP.234.V10	263	9.1	7.1	49	1818	4
1097.05	10	IMYSAHDTTV	PAP.284	217	1.5	14	411	--	4
1389.06	10	ILYSAHDTTV	PAP.284.L2	385	1.0	15	1480	5714	3
60.0213	9	TVSGLQMAV	PAP.292.V9	294	12	122	195	5.7	5
1177.02	9	ALDVYNGLL	PAP.299	73	29	256	3083	--	3
1419.64	10	LLPPYASCHV	PAP.306.V10	88	15	16	98	5260	4

-- indicates binding affinity >10,000nM.

Table XXIIIB A2 supermotif cross-reactive binding data

Peptide	AA	Sequence	Source	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	A2 Cross-Reactivity
1126.10	9	VLAGGFFLL	PSM.27	39	0.20	33	31	2857	4
1389.20	9	VLAGGFFLV	PSM.27.V9	26	0.40	5.0	57	216	5
1129.04	10	GMPEGDLVYV	PSM.168	55	3.1	7.1	161	6154	4
1389.22	10	GLPEGDLVYV	PSM.168.L2	42	2.0	2.1	112	964	4
1418.29	10	GTPEGDLVYV	PSM.168.T2	313	134	53	40	571	4
1129.10	10	GLPSIPVHPI	PSM.288	147	2.7	2.1	2467	308	4
1389.24	10	GLPSIPVHPV	PSM.288.V10	55	0.70	0.60	308	121	5
1129.01	10	LLQERGVAYI	PSM.441	179	5.7	6.7	861	–	3
1126.14	9	LMYSLVHNL	PSM.469	64	0.40	2.1	109	320	5
1126.06	10	RMMNDQLMFL	PSM.662	9.8	2.7	7.7	40	–	4
1126.01	9	MMNDQLMFL	PSM.663	11	0.80	1.7	7.6	195	5
1126.16	10	QLMFLERAFI	PSM.667	98	36	91	–	30	4
1129.08	9	ALFDIESKV	PSM.711	85	0.70	1.4	148	8889	4
1418.30	9	ATFDIESKV	PSM.711.T2	238	27	44	82	258	5

– indicates binding affinity >10,000nM.

Table XXIIIC A2 supermotif cross-reactive binding data

Peptide	AA	Sequence	Source	Alternate Source	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	A2 Cross-Reactivity
1419.25	11	VVFLTLSVTWI	PSA.1		385	159	63	2846	--	3
63.0185	11	VVFLTLSVTWV	PSA.1.V11		89	88	71	336	--	4
63.0186	11	FLTLSVTWIGV	PSA.3.V11		6.8	3.0	18	65	114	5
60.0216	9	FLTLSVTWV	PSA.3.V9		53	8.4	8.3	49	--	4
60.0217	9	TLSVTWIGV	PSA.5.V9		26	4.9	40	712	229	4
1419.10	11	VLVHPQWVLTA	PSA.49	HuK2.53	294	7.7	101	2056	--	3
1419.11	11	VLVHPQWVLTV	PSA.49.V11	HuK2.53.V11	11	1.5	16	31	8889	4
63.0109	11	DLMLLRLSEPV	PSA.116.V11	HuK2.120.V11	50	57	29	148	2759	4
63.0014	10	LMLLRLSEPA	PSA.117	HuK2.121	200	17	67	925	5000	3
1418.43	10	LMLLRLSEPV	PSA.117.V10	HuK2.121.V10	114	67	29	25	6154	4
1419.02	9	MLLRLSEPA	PSA.118	HuK2.122	195	745	145	49	--	3
1389.10	9	MLLRLSEPV	PSA.118.V9	HuK2.122.V9	36	36	46	638	421	4
1389.12	11	MLLRLSEPAEV	PSA.118.V11		294	331	115	1762	4444	3
1419.01	8	ALGTTCYA	PSA.143	HuK2.147	15	19	13	561	--	3
1389.14	8	ALGTTCYV	PSA.143.V8	HuK2.147.V8	74	6.4	12	264	--	4
1098.02	10	FLTPKKLQCV	PSA.161		52	8.3	13	755	--	3
990.01	9	KLQCVDLHV	PSA.166		79	205	91	6167	--	3
63.0058	10	KLQCVDLHVV	PSA.166.V10		13	84	9.1	500	--	4
60.0220	9	KVTKFMLCV	PSA.187.V9		69	518	53	128	--	3
1419.17	11	PLVCNGVLQGV	PSA.212.V11	HuK2.216.V11	27	127	19	255	4314	4
1418.55	10	LVCNGVLQGV	PSA.213.V10	HuK2.217.V10	10	2.9	12	5.6	3.5	5

-- indicates binding affinity >10,000nM.

Table XXIIID A2 supermotif cross-reactive binding data

Peptide	AA	Sequence	Source	Alternate Source	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	A2 Cross-Reactivity
1418.13	9	LLLSIALSV	HuK2.4.L2		88	176	147	189	--	4
1418.57	11	ILLSVGCTGAV	HuK2.8.L2		36	33	36	308	--	4
1418.59	11	ITLSVGCTGAV	HuK2.8.T2		294	134	40	206	121	5
1419.05	10	ALSVGCTGAV	HuK2.9		53	75	17	542	--	3
1418.15	9	ALSVGCTGV	HuK2.9.V9		24	17	9.1	264	--	4
1418.35	10	SVGCTGAVPV	HuK2.11.V10		104	287	154	552	216	4
1419.10	11	VLVHPQWVLTA	HuK2.53	PSA.49	294	7.7	101	2056	--	3
1419.11	11	VLVHPQWVLTV	HuK2.53.V11	PSA.49.V11	11	1.6	16	31	9378	4
63.0109	11	DLMLLRLSEPV	HuK2.120.V11	PSA.116.V11	50	57	29	148	2759	4
63.0014	10	LMLLRLSEPA	HuK2.121	PSA.117	200	17	67	925	5000	3
1418.43	10	LMLLRLSEPV	HuK2.121.V10	PSA.117.V10	114	67	29	25	6154	4
1419.02	9	MLLRLSEPA	HuK2.122	PSA.118	195	745	145	49	--	3
1389.10	9	MLLRLSEPV	HuK2.122.V9	PSA.118.V9	36	36	46	638	421	4
1419.01	8	ALGTTCYA	HuK2.147	PSA.143	15	19	13	561	--	3
1389.14	8	ALGTTCYV	HuK2.147.V8	PSA.143.V8	74	6.4	12	264	--	4
1419.07	10	FLRPRSLQCV	HuK2.165		186	4.8	4.2	--	--	3
60.0191	9	SLQCVSLHL	HuK2.170		500	51	417	6167	2581	3
1419.66	10	SLQCVSLHLL	HuK2.170		263	4.9	71	446	5000	4
1418.52	10	SLQCVSLHLV	HuK2.170.V10		13	6.3	2.8	5.2	205	5
1418.19	9	SLQCVSLHV	HuK2.170.V9		56	165	48	4111	1600	3
1419.14	11	SLHLLSNDMCA	HuK2.175		71	4.8	71	--	--	3
1418.66	11	SLHLLSNDMCV	HuK2.175.V11		8.6	0.80	10	2313	2162	3
1419.15	11	HLLSNDMCARA	HuK2.177		417	391	250	374	--	4
1418.67	11	HLLSNDMCARV	HuK2.177.V11		26	1.3	5.3	37	860	4
1418.20	9	HLLSNDMCV	HuK2.177.V9		119	102	278	176	--	4
1418.53	10	LLSNDMCARV	HuK2.178.V10		5.3	0.70	4.3	10	1702	4
1418.71	11	KVTEFMLCAGV	HuK2.191.V11		56	10	26	29	143	5
1418.21	9	KVTEFMLCV	HuK2.191.V9		53	27	31	34	6667	4
1418.22	9	FMLCAGLWV	HuK2.195.V9		29	12	91	51	--	4
1419.17	11	PLVCNGVLQGV	HuK2.216.V11	PSA.212.V11	27	127	19	255	4314	4
1418.55	10	LVCNGVLQGV	HuK2.217.V10	PSA.213.V11	10	2.9	12	5.6	3.5	5

-- indicates binding affinity >10,000nM.

Table XXIVA Immunogenicity of A2 cross-reactive binding peptides and peptide analogs

Peptide ID	AA	Sequence	Source	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	Cross-Reactivity (≤200nM)	A2 peptide	A2 native	A2 in vivo
1419.51	10	SLSLGFLFLL	PAP.13	40	13	403	21	8560	3			
1419.52	10	SLSLGFLFLV	PAP.13.V10	1.8	3.9	17	42	355	4			
1097.09	10	LLFFWLDRSV	PAP.21	28	0.60	1.6	231	--	3	3/3		0/3
1418.23	10	LTFFWLDRSV	PAP.21.T2	118	11	9.6	43	16	5	3/3	2/3	
1097.17	9	VLAKELKFV	PAP.30	96	3.6	6.7	168	--	4	1/3		0/3
1177.01	9	TLMSAMTNL	PAP.112	43	0.80	2.9	285	296	3	2/2		3/3
1419.58	10	LLALFPPEGV	PAP.120.L2	5.0	0.72	1.6	146	164	5			
1419.61	10	ALFPPEGVSV	PAP.122.V10	15	1.0	18	120	4387	4	1/3	1/3	
1044.04	9	ILLWQPIPV	PAP.135	3.3	39	1.8	71	8511	4	5/5		1/6
1418.25	9	ITLWQPIPV	PAP.135.T2	34	1723	6.2	26	32	4	3/3	2/3	
1419.69	10	LLWQPIPVHV	PAP.136.V10	25	1.8	17	287	60	4			
1166.11	10	GLHGQDLFGI	PAP.196	26	0.9	2.5	315	--	3			
1419.62	10	GLHGQDLFGV	PAP.196.V10	12	2.3	3.2	18	--	4			
1097.05	10	IMYSAHDTTV	PAP.284	217	1.5	14	411	--	2	3/3		0/3
1419.64	10	LLPPYASCHV	PAP.306.V10	88	15	16	98	5260	4			

Table XXIVB Immunogenicity of A2 cross-reactive binding peptide and peptide analogs

Peptide ID	AA	Sequence	Source	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	Cross-Reactivity (≤200nM)	A2 peptide	A2 native	A2 in vivo
1126.10	9	VLAGGFFLL	PSM.27	39	0.20	33	31	--	4	1/2		3/3
1389.20	9	VLAGGFFLV	PSM.27.V9	26	0.40	5.0	57	216	4	1/2	1/2	
1129.04	10	GMPEGDLVYV	PSM.168	55	3.1	7.1	161	--	4	0/1		1/3
1129.10	10	GLPSIPVHPI	PSM.288	147	2.7	2.1	2467	1538	3	2/4		0/3
1389.24	10	GLPSIPVHPV	PSM.288.V10	55	0.70	0.60	308	121	4	4/4	3/4	
1129.01	10	LLQERGVAYI	PSM.441	179	5.7	6.7	861	--	3	3/3		
1126.14	9	LMYSLVHNL	PSM.469	64	0.40	2.1	109	1600	4	3/3		3/3
1126.06	10	RMMNDQLMFL	PSM.662	9.8	2.7	7.7	40	--	4	1/1		20/22
1126.01	9	MMNDQLMFL	PSM.663	11	0.80	1.7	7.6	976	4	2/2		3/3
1129.08	9	ALFDIESKV	PSM.711	85	0.70	1.4	148	--	4	2/2		3/3

Table XXIVD Immunogenicity of A2 cross-reactive binding peptides and peptide analogs

Peptide	ID	AA	Sequence	Source	Alternate Source	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	Cross-Reactivity (<200nM)	A2 peptide	A2 native	A2 in vivo
1418.13		9	LLLSIALSV	HuK2.4.L2		88	176	147	189	--	4	2/2	2/2	
1419.05		10	ALSVGCTGAV	HuK2.9		53	75	17	542	--	3			
1419.11		11	VLVHPQWVLTV	HuK2.53.V11	PSA 49.V11	11	1.6	16	31.	9378	4	2/2	2/2	
1419.13		11	DLMLLRLSEPV	HuK2.120.V11	PSA.116.V11	50	57	29	148	2759	4	2/2	2/2	
1419.02		9	MLLRLSEPA	HuK2.122	PSA.118	195	745	145	49	--	3			
1389.10		9	MLLRLSEPV	HuK2.122.V9	PSA.118.V9	36	36	46	638	421	3			
1419.01		8	ALGTTCYA	HuK2.147	PSA.143	15	19	13	562	--	3	1/2		
1389.14		8	ALGTTCYV	HuK2.147.V8	PSA.143.V8	74	6.4	12	264	--	3			
1419.07		10	FLRPRSLQCV	HuK2.165		186	4.8	4	--	--	3	1/3		
1419.14		11	SLHLLSNDMCA	HuK2.175		72	4.8	73	--	--	3	1/3		
1419.17		11	PLVCNGVLQGV	HuK2.216.V11	PSA.212.V11	27	127	19	255	4314	3	2/2	2/2	

Table XXIVC Immunogenicity of A2 cross-reactive binding peptides and peptide analogs

Peptide ID	AA	Sequence	Source	Alternate Source	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	Cross-Reactivity (≤200nM)	A2 peptide	A2 native	A2 in vivo
1419.27	11	FLTLSVTWIGV	PSA.3.V11		6.8	3.0	18	65	113	5	3/3	3/3	
1419.11	11	VLVHPQWVLTV	PSA 49.V11	HuK2.53.V11	11	1.6	16	31	9378	4			
1419.13	11	DLMLLRLSEPV	PSA.116.V11	HuK2.120.V11	50	57	29	148	2759	4			
1419.02	9	MLLRLSEPA	PSA.118	HuK2.122	195	745	145	49	--	3			
1389.10	9	MLLRLSEPV	PSA.118.V9	HuK2.122.V9	36	36	46	638	421	3	3/3	1/3	
1419.01	8	ALGTTCYA	PSA.143	PSA.143	15	19	13	562	--	3			
1389.14	8	ALGTTCYV	PSA.143.V8	HuK2.147.V8	74	6.4	12	264	--	3	2/3	1/3	
1098.02	10	FLTPKKLQCV	PSA.161		52	8.3	13	755	--	3	3/4		0/6
990.01	9	KLQCVDLHV	PSA.166		79	205	91	6167	--	2	1/2		1/3
1419.24	10	KLQCVDLHVV	PSA.166.V10		13	84	9.5	502	--	3	1/2	1/2	
1419.17	11	PLVCNGVLQGV	PSA.212.V11	HuK2.216.V11	27	127	19	255	4314	3			

Table XXV.
DR supermotif and DR3 motif-bearing peptides
cross-reactive binding peptides

	DR supermotif		DR3
Antigen	Motif+	Algorithm+*	Motif+
PAP	67	39/15	21
PSM	45	25/7	4
PSA	108	54/20	31
HuK2	45	21/6	4
Total	265	139/48	60

*Number scoring positive in the combined DR1, DR4w4 and DR7 algorithms (≥1/≥2)

WHAT IS CLAIMED IS:

1. An isolated prepared prostate cancer-associated antigen epitope consisting of a sequence selected from the group consisting of the sequences set out in Table XXIV.

2. A composition of claim 1, wherein the epitope is admixed or joined to a CTL epitope.

3. A composition of claim 2, wherein the CTL epitope is selected from the group set out in claim 1.

4. A composition of claim 1, wherein the epitope is admixed or joined to an HTL epitope.

5. A composition of claim 4, wherein the HTL epitope is selected from the group set out in claim 1.

6. A composition of claim 4, wherein the HTL epitope is a pan-DR binding molecule.

7. A composition of claim 1, comprising at least three epitopes selected from the group set out in claim 1.

8. A composition of claim 1, further comprising a liposome, wherein the epitope is on or within the liposome.

9. A composition of claim 1, wherein the epitope is joined to a lipid.

10. A composition of claim 1, wherein the epitope is joined to a linker.

11. A composition of claim 1, wherein the epitope is bound to an HLA heavy chain, β2-microglobulin, and strepavidin complex, whereby a tetramer is formed.

12. A composition of claim 1, further comprising an antigen presenting cell, wherein the epitope is on or within the antigen presenting cell.

13. A composition of claim 12, wherein the epitope is bound to an HLA molecule on the antigen presenting cell, whereby when a cytotoxic lymphocyte (CTL) is present that is restricted to the HLA molecule, a receptor of the CTL binds to a complex of the HLA molecule and the epitope.

14. A clonal cytotoxic T lymphocyte (CTL), wherein the CTL is cultured *in vitro* and binds to a complex of an epitope selected from the group set out in Table XXIV, bound to an HLA molecule.

15. A peptide comprising at least a first and a second epitope, wherein the first epitope is selected from the group consisting of the sequences set out in Table XXIV;

wherein the peptide comprise less than 50 contiguous amino acids that have 100% identity with a native peptide sequence.

16. A composition of claim 15, wherein the first and the second epitope are selected from the group of claim 14.

17. A composition of claim 16, further comprising a third epitope selected from the group of claim 15.

18. A composition of claim 15, wherein the peptide is a heteropolymer.

19. A composition of claim 15, wherein the peptide is a homopolymer.

20. A composition of claim 15, wherein the second epitope is a CTL epitope.

21. A composition of claim 20, wherein the CTL epitope is from a tumor associated antigen that is not prostate specific antigen (PSA), prostate specific membrane antigen (PSM), prostatic acid phosphatase (PAP), or human kallikrein2 (HuK2).

22. A composition of claim 15, wherein the second epitope is a PanDR binding molecule.

23. A composition of claim 1, wherein the first epitope is linked to an a linker sequence.

24. A vaccine composition comprising:

a unit dose of a peptide that comprises less than 50 contiguous amino acids that have 100% identity with a native peptide sequence of a prostate cancer-associated antigen, the peptide comprising at least a first epitope selected from the group consisting of the sequences set out in Table XXIV; and;

a pharmaceutical excipient.

25. A vaccine composition in accordance with claim 24, further comprising a second epitope.

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13*ter* and 39)

Applicant's or agent's file reference 18623-1472PC	IMPORTANT DECLARATION	Date of mailing *(day/month/year)* 09 MAY 2001
International application No. PCT/US00/35516	International filing date *(day/month/year)* 20 DECEMBER 2000	(Earliest) Priority Date *(day/month/year)* 21 DECEMBER 1999
International Patent Classification (IPC) or both national classification and IPC Please See Continuation Sheet.		
Applicant EPIMMUNE INC.		

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

1. ☐ The subject matter of the international application relates to:
   - a. ☐ scientific theories.
   - b. ☐ mathematical theories.
   - c. ☐ plant varieties.
   - d. ☐ animal varieties.
   - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
   - f. ☐ schemes, rules or methods of doing business.
   - g. ☐ schemes, rules or methods of performing purely mental acts.
   - h. ☐ schemes, rules or methods of playing games.
   - i. ☐ methods for treatment of the human body by surgery or therapy.
   - j. ☐ methods for treatment of the animal body by surgery or therapy.
   - k. ☐ diagnostic methods practiced on the human or animal body.
   - l. ☐ mere presentations of information.
   - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.

2. ☒ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:
   ☒ the description ☒ the claims ☐ the drawings

3. ☐ The failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions prevents a meaningful search from being carried out.
   - ☐ the written form has not been furnished or does not comply with the standard.
   - ☐ the computer readable form has not been furnished or does not comply with the standard.

4. Further comments:
   Please See Continuation Sheet.

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ANNE L. HOLLERAN TERRY J. DEY PARALEGAL SPECIALIST TECHNOLOGY CENTER 1600
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/203 (July 1998)★

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

l.....al application No.
PCT/US00/35516

The International Patent Classification (IPC) or National Classification and IPC are as listed below:

IPC(7): A61K 38/08, 39/00, 39/385, 39/39; C07H 21/04; C07K 14/435 US Cl: 424/184.1, 185.1; 530/ 300, 328; 536/23.4; 23.5

4. Further Comments (Continued):

No meaningful search could be performed for the claims, 1-34, because there is no correlation in the claims, sequence listing or disclosure between the sequences recited in the 1-letter code of Tables XXIVA-D, and the sequences recited in the 3-letter coled in the sequence listing. It is, further, not clear that the Table XXIV referred to in claims is the same as Tables XXIVA-D. Careful review of the sequences listed on page 15 and in Table IV, of the description, did not reveal the required correlation. Text appears to be missing following "SEQ ID NO: " in Table IV. PCT Rule 62(a) states that the claims shall not rely on references to description or drawings.